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(54) Title: LEPIDOPTERAN-ACTIVE *BACILLUS THURINGIENSIS* δ-ENDOTOXIN COMPOSITIONS AND METHODS OF USE

(57) Abstract: Disclosed are *Bacillus thuringiensis* strains comprising novel crystal proteins which exhibit insecticidal activity against lepidopteran insects. Also disclosed are novel *B. thuringiensis* genes and their encoded crystal proteins, as well as methods of making and using transgenic cells comprising the novel nucleic acid sequences of the invention.

Lepidopteran-Active *Bacillus thuringiensis* δ-Endotoxin Compositions and Methods of Use

1.0 Background of the Invention

1.1 Field of the Invention

The present invention relates generally to the fields of molecular biology. More particularly, certain embodiments concern methods and compositions comprising DNA segments, and proteins derived from bacterial species. More particularly, it concerns novel genes from *Bacillus thuringiensis* encoding lepidopteran-toxic crystal proteins. Various methods for making and using these DNA segments, DNA segments encoding synthetically-modified Cry proteins, and native and synthetic crystal proteins are disclosed, such as, for example, the use of DNA segments as diagnostic probes and templates for protein production, and the use of proteins, fusion protein carriers and peptides in various immunological and diagnostic applications. Also disclosed are methods of making and using nucleic acid segments in the development of transgenic plant cells containing the DNA segments disclosed herein.

1.2 Description of the Related Art

Almost all field crops, plants, and commercial farming areas are susceptible to attack by one or more insect pests. Particularly problematic are Coleopteran and Lepidoptern pests. For example, vegetable and cole crops such as artichokes, kohlrabi, arugula, leeks, asparagus, lentils, beans, lettuce (*e.g.,* head, leaf, romaine), beets, bok choy, malanga, broccoli, melons (*e.g.,* muskmelon, watermelon, crenshaw, honeydew,cantaloupe), brussels sprouts, cabbage, cardoni, carrots, napa, cauliflower, okra, onions, celery, parsley, chick peas, parsnips, chicory, peas, chinese cabbage, peppers, collards, potatoes, cucumber, pumpkins, cucurbits, radishes, dry bulb onions, rutabaga, eggplant, salsify, escarole, shallots, endive, soybean, garlic, spinach, green onions, squash, greens, sugar beets, sweet potatoes, turnip, swiss chard, horseradish, tomatoes, kale, turnips, and a variety of spices are sensitive to infestation by one or more of the following insect pests: alfalfa looper, armyworm, beet armyworm, artichoke plume moth, cabbage budworm, cabbage looper, cabbage webworm, corn earworm, celery leafeater, cross-striped cabbageworm, european corn borer, diamondback moth, green cloverworm, imported cabbageworm, melonworm, omnivorous leafroller, pickleworm, rindworm complex, saltmarsh caterpillar, soybean looper, tobacco budworm, tomato fruitworm, tomato hornworm, tomato

pinworm, velvetbean caterpillar, and yellowstriped armyworm. Likewise, pasture and hay crops such as alfalfa, pasture grasses and silage are often attacked by such pests as armyworm, beef armyworm, alfalfa caterpillar, European skipper, a variety of loopers and webworms, as well as yellowstriped armyworms.

Fruit and vine crops such as apples, apricots, cherries, nectarines, peaches, pears, plums, prunes, quince almonds, chestnuts, filberts, pecans, pistachios, walnuts, citrus, blackberries, blueberries, boysenberries, cranberries, currants, loganberries, raspberries, strawberries, grapes, avocados, bananas, kiwi, persimmons, pomegranate, pineapple, tropical fruits are often susceptible to attack and defoliation by achema sphinx moth, amorbia, armyworm, citrus cutworm, banana skipper, blackheaded fireworm, blueberry leafroller, cankerworm, cherry fruitworm, citrus cutworm, cranberry girdler, eastern tent caterpillar, fall webworm, fall webworm, filbert leafroller, filbert webworm, fruit tree leafroller, grape berry moth, grape leaffolder, grapeleaf skeletonizer, green fruitworm, gummosos-batrachedra commosae, gypsy moth, hickory shuckworm, hornworms, loopers, navel orangeworm, obliquebanded leafroller, omnivorous leafroller. omnivorous looper, orange tortrix, orangedog, oriental fruit moth, pandemis leafroller, peach twig borer, pecan nut casebearer, redbanded leafroller, redhumped caterpillar, roughskinned cutworm, saltmarsh caterpillar, spanworm, tent caterpillar, thecla-thecla basillides, tobacco budworm, tortrix moth, tufted apple budmoth, variegated leafroller, walnut caterpillar, western tent caterpillar, and yellowstriped armyworm.

Field crops such as canola/rape seed, evening primrose, meadow foam, corn (field, sweet, popcorn), cotton, hops, jojoba, peanuts, rice, safflower, small grains (barley, oats, rye, wheat, *etc.*), sorghum, soybeans, sunflowers, and tobacco are often targets for infestation by insects including armyworm, asian and other corn borers, banded sunflower moth, beet armyworm, bollworm, cabbage looper, corn rootworm (including southern and western varieties), cotton leaf perforator, diamondback moth, european corn borer, green cloverworm, headmoth, headworm, imported cabbageworm, loopers (including *Anacamptodes* spp.), obliquebanded leafroller, omnivorous leaftier, podworm, podworm, saltmarsh caterpillar, southwestern corn borer, soybean looper, spotted cutworm, sunflower moth, tobacco budworm, tobacco hornworm, velvetbean caterpillar.

Bedding plants, flowers, ornamentals, vegetables and container stock are frequently fed upon by a host of insect pests such as armyworm, azalea moth, beet armyworm, diamondback

moth, ello moth (hornworm), Florida fern caterpillar, Io moth, loopers, oleander moth, omnivorous leafroller, omnivorous looper, and tobacco budworm.

Forests, fruit, ornamental, and nut-bearing trees, as well as shrubs and other nursery stock are often susceptible to attack from diverse insects such as bagworm, blackheaded budworm, browntail moth, california oakworm, douglas fir tussock moth, elm spanworm, fall webworm, fruittree leafroller, greenstriped mapleworm, gypsy moth, jack pine budworm, mimosa webworm, pine butterfly, redhumped caterpillar, saddleback caterpillar, saddle prominent caterpillar, spring and fall cankerworm, spruce budworm, tent caterpillar, tortrix, and western tussock moth. Likewise, turf grasses are often attacked by pests such as armyworm, sod webworm, and tropical sod webworm.

Because crops of commercial interest are often the target of insect attack, environmentally-sensitive methods for controlling or eradicating insect infestation are desirable in many instances. This is particularly true for farmers, nurserymen, growers, and commercial and residential areas which seek to control insect populations using eco-friendly compositions.

The most widely used environmentally-sensitive insecticidal formulations developed in recent years have been composed of microbial pesticides derived from the bacterium *Bacillus thuringiensis*. *B. thuringiensis* is a Gram-positive bacterium that produces crystal proteins or inclusion bodies which are specifically toxic to certain orders and species of insects. Many different strains of *B. thuringiensis* have been shown to produce insecticidal crystal proteins. Compositions including *B. thuringiensis* strains which produce insecticidal proteins have been commercially-available and used as environmentally-acceptable insecticides because they are quite toxic to the specific target insect, but are harmless to plants and other non-targeted organisms.

1.2.1 *B. thuringiensis* Crystal Proteins δ-ENDOTOXINS

δ-endotoxins are used to control a wide range of leaf-eating caterpillars and beetles, as well as mosquitoes. These proteinaceous parasporal crystals, also referred to as insecticidal crystal proteins, crystal proteins, Bt inclusions, crystaline inclusions, inclusion bodies, and Bt toxins, are a large collection of insecticidal proteins produced by *B. thuringiensis* that are toxic upon ingestion by a susceptible insect host. Over the past decade research on the structure and function of *B. thuringiensis* toxins has covered all of the major toxin categories, and while these

toxins differ in specific structure and function, general similarities in the structure and function are assumed. Based on the accumulated knowledge of *B. thuringiensis* toxins, a generalized mode of action for *B. thuringiensis* toxins has been created and includes: ingestion by the insect, solubilization in the insect midgut (a combination stomach and small intestine), resistance to digestive enzymes sometimes with partial digestion actually "activating" the toxin, binding to the midgut cells, formation of a pore in the insect cells and the disruption of cellular homeostasis (English and Slatin, 1992).

One of the unique features of *B. thuringiensis* is its production of crystal proteins during sporulation which are specifically toxic to certain orders and species of insects. Many different strains of *B. thuringiensis* have been shown to produce insecticidal crystal proteins. Compositions including *B. thuringiensis* strains which produce proteins having insecticidal activity against lepidopteran and dipteran insects have been commercially available and used as environmentally-acceptable insecticides because they are quite toxic to the specific target insect, but are harmless to plants and other non-targeted organisms.

The mechanism of insecticidal activity of the *B. thuringiensis* crystal proteins has been studied extensively in the past decade. It has been shown that the crystal proteins are toxic to the insect only after ingestion of the protein by the insect. The alkaline pH and proteolytic enzymes in the insect mid-gut solubilize the proteins, thereby allowing the release of components which are toxic to the insect. These toxic components disrupt the mid-gut cells, cause the insect to cease feeding, and, eventually, bring about insect death. For this reason, *B. thuringiensis* has proven to be an effective and environmentally safe insecticide in dealing with various insect pests.

As noted by Höfte and Whiteley (1989), the majority of insecticidal *B. thuringiensis* strains are active against insects of the order Lepidoptera, *i.e.*, caterpillar insects. Other *B. thuringiensis* strains are insecticidally active against insects of the order Diptera, *i.e.*, flies and mosquitoes, or against both lepidopteran and dipteran insects. In recent years, a few *B. thuringiensis* strains have been reported as producing crystal proteins that are toxic to insects of the order Coleoptera, *i.e.*, beetles (Krieg *et al.*, 1983; Sick *et al.*, 1990; Donovan *et al.*, 1992; Lambert *et al.*, 1992a; 1992b).

1.2.2 Genes Encoding Crystal Proteins

Many of the δ-endotoxins are related to various degrees by similarities in their amino acid sequences. Historically, the proteins and the genes which encode them were classified based largely upon their spectrum of insecticidal activity. The review by Höfte and Whiteley (1989) discusses the genes and proteins that were identified in *B. thuringiensis* prior to 1990, and sets forth the nomenclature and classification scheme which has traditionally been applied to *B. thuringiensis* genes and proteins. *cryI* genes encode lepidopteran-toxic CryI proteins. *cryII* genes encode CryII proteins that are toxic to both lepidopterans and dipterans. *cryIII* genes encode coleopteran-toxic CryIII proteins, while *cryIV* genes encode dipteran-toxic CryIV proteins. Based on the degree of sequence similarity, the proteins were further classified into subfamilies; more highly related proteins within each family were assigned divisional letters such as CryIA, CryIB, CryIC, *etc.* Even more closely related proteins within each division were given names such as CryIC1, CryIC2, *etc.*

Recently, a new nomenclature was developed which systematically classified the Cry proteins based upon amino acid sequence homology rather than upon insect target specificities (Crickmore *et al.*, 1998). The classification scheme for many known toxins, including allelic variations in individual proteins, is summarized and regularly updated at http://www.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/. The informationw was most recently updated as of April 27, 1999 and is herein incorporated by reference.

1.2.3 Crystal Proteins Toxic to Lepidopteran Insects

2.0 Summary of the Invention

The recent review by Schnepf *et al.* (1998) describes the enormous diversity of insecticidal crystal proteins derived from *B. thuringiensis*. Cry proteins of the Cry1, Cry2, and Cry9 classes are particularly known for their toxicity towards lepidopteran larvae, however, the degree of toxicity varies significantly depending on the target lepidopteran pest (Höfte and Whiteley, 1989). For instance, Cry1Ac shows poor toxicity towards the armyworm, *Spodoptera littoralis*, but strong toxicity towards the tobacco budworm, *Heliothis virescens*. In addition, slight variations in amino acid sequence within a Cry protein class can dramatically impact insecticidal activity (see Schnepf *et al.*, 1998 and references therein). The Cry3Ba and Cry3Bb genes, for instance, share 94% amino acid sequence identity, but only Cry3Bb exhibits

significant toxicity towards the Southern corn rootwom, *Diabrotica undecimpunctata howardi* (Donovan *et al.*, 1992). Similarly, Cry2Aa and Cry2Ab share 87% amino acid sequence identity, yet only Cry2Aa displays toxicity towards mosquitos (Widner and Whiteley, 1990). Von Tersch *et al.* (1991) demonstrated that Cry1Ac proteins varying by only seven amino acids (>99% sequence identity) nevertheless show significant differences in insecticidal activity. Lee *et al.* (1996) reported that Cry1Ab alleles differing at only two amino acid positions exhibited a 10-fold difference in toxicity towards the gypsy moth, *Lymantria dispar*. Thus, even Cry proteins that are considered to be alleles of known Cry proteins or to belong to a Cry protein subclass (Crickmore *et al.*, 1998) may have unique and useful insecticidal properties. International Patent Application Publication No. WO 98/00546 and WO 98/40490 describe a variety of Cry1-, Cry2-, and Cry9-related crystal proteins obtained from *B. thuringiensis*.

2.1 *Cry* DNA Segments

The present invention concerns nucleic acid segments, that can be isolated from virtually any source, that are free from total genomic DNA and that encode the novel peptides disclosed herein. Nucleic acid segments encoding these polypeptides may encode active proteins, peptides or peptide fragments, polypeptide subunits, functional domains, or the like of one or more crystal proteins. In addition the invention encompasses nucleic acid segments which may be synthesized entirely *in vitro* using methods that are well-known to those of skill in the art which encode the novel Cry polypeptides, peptides, peptide fragments, subunits, or functional domains disclosed herein.

As used herein, the term "nucleic acid segment" refers to a polynucleotide molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a nucleic acid segment encoding an endotoxin polypeptide refers to a nucleic acid segment that comprises one or more crystal protein-encoding sequences yet is isolated away from, or purified free from, total genomic DNA of the species from which the nucleic acid segment is obtained, which in the instant case is the genome of the Gram-positive bacterial genus, *Bacillus*, and in particular, the species of *Bacillus* known as *B. thuringiensis*. Included within the term "nucleic acid segment", are polynucleotide segments and smaller fragments of such segments, and also recombinant vectors, including, for example, plasmids, cosmids, phagemids, phages, viruses, and the like.

Similarly, a DNA segment comprising an isolated or purified crystal protein-encoding gene refers to a DNA segment which may include in addition to peptide encoding sequences,

certain other elements such as, regulatory sequences, isolated substantially away from other naturally occurring genes or protein-encoding sequences. In this respect, the term "gene" is used for simplicity to refer to a functional protein-, polypeptide- or peptide-encoding unit. As will be understood by those in the art, this functional term includes both genomic sequences, operon sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides or peptides. Also, the term includes an expression cassette comprising at least a promoter operably linked to one or more protein coding sequences, operably linked to at least a transcriptional termination sequence.

"Isolated substantially away from other coding sequences" means that the gene of interest, in this case, a nucleic acid segment or gene encoding all or part of a bacterial insecticidal crystal protein, forms the significant part of the coding region of the DNA segment, and that the DNA segment does not contain large portions of naturally-occurring coding DNA, such as large chromosomal fragments or other functional nucleic acid segments or genes or operon coding regions. Of course, this refers to the DNA segment as originally isolated, and does not exclude genes, recombinant genes, synthetic linkers, or coding regions later added to the segment by the hand of man.

In particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences that encode a Cry peptide species that includes within its amino acid sequence an amino acid sequence essentially as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50 and SEQ ID NO. 63.

The term "a sequence essentially as set forth in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6," for example, means that the sequence substantially corresponds to a portion of the sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6 and has relatively few amino acids that are not identitical with, or a biologically functional equivalent of, the amino acids of any of these sequences. The term "biologically functional equivalent" is well understood in the art and is further defined in detail herein (*e.g.*, see Illustrative Embodiments). Accordingly, sequences that have from about 70% to about 80%, or more preferably about 81, 82, 83, 84, 85, 86, 87, 88,

89, or about 90%, or even more preferably about 91, 92, 93, 94, 95, 96, 97, 98, or about 99% amino acid sequence identity or functional equivalence to the amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50 and SEQ ID NO. 63 will be sequences that are "essentially as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50 and SEQ ID NO: 63."

In addition, sequences that have from about 70% to about 80%, or more preferably about 81, 82, 83, 84, 85, 86, 87, 88, 89, or about 90%, or even more preferably about 91, 92, 93, 94, 95, 96, 97, 98, or about 99% nucleic acid sequence identity or functional equivalence to the nucleic acids of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49 and SEQ ID NO:62 will be sequences that are "essentially as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49 and SEQ ID NO:62."

It will also be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids or 5′ or 3′ sequences, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein activity where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences

flanking either of the 5′ or 3′ portions of the coding region or may include various internal sequences, *i.e.*, introns, which are known to occur within genes.

The nucleic acid segments of the present invention, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, nucleic acid fragments may be prepared that include a short contiguous stretch encoding any of the peptide sequences disclosed in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50 and SEQ ID NO: 63, or that are identical with or complementary to DNA sequences which encode any of the peptides disclosed in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50 and SEQ ID NO: 63, and particularly those DNA segments disclosed in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49 and SEQ ID NO:62. For example, DNA sequences such as about 18 nucleotides, and that are up to about 10,000, about 5,000, about 3,000, about 2,000, about 1,000, about 500, about 200, about 100, about 50, and about 14 base pairs in length (including all intermediate lengths) are also contemplated to be useful.

It will be readily understood that "intermediate lengths", in these contexts, means any length between the quoted ranges, such as 18, 19, 20, 21, 22, 23, *etc.*; 30, 31, 32, *etc.*; 50, 51, 52,

53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers in the ranges of from about 200-500; 500-1,000; 1,000-2,000; 2,000-3,000; 3,000-5,000; and up to and including sequences of about 10,00 or so nucleotides and the like.

It will also be understood that this invention is not limited to the particular nucleic acid sequences which encode peptides of the present invention, or which encode the amino acid sequences of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50 and SEQ ID NO: 63, including those DNA sequences which are particularly disclosed in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49 and SEQ ID NO:62. Recombinant vectors and isolated DNA segments may therefore variously include the peptide-coding regions themselves, coding regions bearing selected alterations or modifications in the basic coding region, or they may encode larger polypeptides that nevertheless include these peptide-coding regions or may encode biologically functional equivalent proteins or peptides that have variant amino acids sequences.

The DNA segments of the present invention encompass biologically-functional, equivalent peptides. Such sequences may arise as a consequence of codon degeneracy and functional equivalency that are known to occur naturally within nucleic acid sequences and the proteins thus encoded. Alternatively, functionally-equivalent proteins or peptides may be created via the application of recombinant DNA technology, in which changes in the protein structure may be engineered, based on considerations of the properties of the amino acids being exchanged. Changes designed by man may be introduced through the application of site-directed mutagenesis techniques, *e.g.*, to introduce improvements to the antigenicity of the protein or to test mutants in order to examine activity at the molecular level.

If desired, one may also prepare fusion proteins and peptides, *e.g.*, where the peptide-coding regions are aligned within the same expression unit with other proteins or peptides having

desired functions, such as for purification or immunodetection purposes (*e.g.*, proteins that may be purified by affinity chromatography and enzyme label coding regions, respectively).

Recombinant vectors form further aspects of the present invention. Particularly useful vectors are contemplated to be those vectors in which the coding portion of the DNA segment, whether encoding a full length protein or smaller peptide, is positioned under the control of a promoter. The promoter may be in the form of the promoter that is naturally associated with a gene encoding peptides of the present invention, as may be obtained by isolating the 5′ non-coding sequences located upstream of the coding segment or exon, for example, using recombinant cloning and/or PCR™ technology, in connection with the compositions disclosed herein.

2.2 *Cry* DNA Segments as Hybridization Probes And Primers

In addition to their use in directing the expression of crystal proteins or peptides of the present invention, the nucleic acid sequences contemplated herein also have a variety of other uses. For example, they also have utility as probes or primers in nucleic acid hybridization embodiments. As such, it is contemplated that nucleic acid segments that comprise a sequence region that consists of at least a 14 nucleotide long contiguous sequence that has the same sequence as, or is complementary to, a 14 nucleotide long contiguous DNA segment of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49 and SEQ ID NO:62 will find particular utility. Longer contiguous identical or complementary sequences, *e.g.*, those of about 20, 30, 40, 50, 100, 200, 500, 1000, 2000, 5000 bp, *etc.* (including all intermediate lengths and up to and including the full-length gene sequences encoding each polypeptide will also be of use in certain embodiments.

The ability of such nucleic acid probes to specifically hybridize to crystal protein-encoding sequences will enable them to be of use in detecting the presence of complementary sequences in a given sample. However, other uses are envisioned, including the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

Nucleic acid molecules having sequence regions consisting of contiguous nucleotide stretches of about 14 to about 17 or so, 18-25, 26-35, 36-50, or even up to and including sequences of about 100-200 nucleotides or so, identical or complementary to DNA sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49 and SEQ ID NO:62, are particularly contemplated as hybridization probes for use in, *e.g.*, Southern and Northern blotting. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the contiguous complementary region may be varied, such as between about 10-14 and about 100 to 200 or so nucleotides, but larger contiguous complementarity stretches may be used, according to the length complementary sequences one wishes to detect.

Of course, fragments may also be obtained by other techniques such as, *e.g.*, by mechanical shearing or by restriction enzyme digestion. Small nucleic acid segments or fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer. Also, fragments may be obtained by application of nucleic acid reproduction technology, such as the PCR™ technology of U. S. Patents 4,683,195 and 4,683,202 (each incorporated herein by reference), by introducing selected sequences into recombinant vectors for recombinant production, and by other recombinant DNA techniques generally known to those of skill in the art of molecular biology.

Accordingly, the nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of DNA fragments. Depending on the application envisioned, one will desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of probe towards target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, *e.g.*, one will select relatively low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C. Such selective conditions tolerate little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating crystal protein-encoding DNA

segments. Detection of DNA segments via hybridization is well-known to those of skill in the art, and the teachings of U. S. Patents 4,965,188 and 5,176,995 (each incorporated herein by reference) are exemplary of the methods of hybridization analyses. Teachings such as those found in the texts of Maloy *et al.*, 1990; Maloy 1994; Segal, 1976; Prokop, 1991; and Kuby, 1991, are particularly relevant.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template or where one seeks to isolate crystal protein-encoding sequences from related species, functional equivalents, or the like, less stringent hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ conditions such as about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

In certain embodiments, it will be advantageous to employ nucleic acid sequences of the present invention in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including fluorescent, radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal. In preferred embodiments, one will likely desire to employ a fluorescent label or an enzyme tag, such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmentally undesirable reagents. In the case of enzyme tags, colorimetric indicator substrates are known that can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with complementary nucleic acid-containing samples.

In general, it is envisioned that the hybridization probes described herein will be useful both as reagents in solution hybridization as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed, single-stranded nucleic acid is then subjected to specific

hybridization with selected probes under desired conditions. The selected conditions will depend on the particular circumstances based on the particular criteria required (depending, for example, on the G+C content, type of target nucleic acid, source of nucleic acid, size of hybridization probe, *etc.*). Following washing of the hybridized surface so as to remove nonspecifically bound probe molecules, specific hybridization is detected, or even quantitated, by means of the label.

2.3 Vectors and Methods for Recombinant Expression of Cry Polypeptides

In other embodiments, it is contemplated that certain advantages will be gained by positioning the coding DNA segment under the control of a recombinant, or heterologous, promoter. As used herein, a recombinant or heterologous promoter is intended to refer to a promoter that is not normally associated with a DNA segment encoding a crystal protein or peptide in its natural environment. Such promoters may include promoters normally associated with other genes, and/or promoters isolated from any bacterial, viral, eukaryotic, or plant cell. Naturally, it will be important to employ a promoter that effectively directs the expression of the DNA segment in the cell type, organism, or even animal, chosen for expression. The use of promoter and cell type combinations for protein expression is generally known to those of skill in the art of molecular biology, for example, see Sambrook *et al.*, 1989. The promoters employed may be constitutive, or inducible, and can be used under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins or peptides. Appropriate promoter systems contemplated for use in high-level expression include, but are not limited to, the *Pichia* expression vector system (Pharmacia LKB Biotechnology).

In connection with expression embodiments to prepare recombinant proteins and peptides, it is contemplated that longer DNA segments will most often be used, with DNA segments encoding the entire peptide sequence being most preferred. However, it will be appreciated that the use of shorter DNA segments to direct the expression of crystal peptides or epitopic core regions, such as may be used to generate anti-crystal protein antibodies, also falls within the scope of the invention. DNA segments that encode peptide antigens from about 8 to about 50 amino acids in length, or more preferably, from about 8 to about 30 amino acids in length, or even more preferably, from about 8 to about 20 amino acids in length are contemplated to be particularly useful. Such peptide epitopes may be amino acid sequences which comprise contiguous amino acid sequences from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID

NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50 and SEQ ID NO: 63.

2.4 *cry* Transgenes and Transgenic Plants Expressing Cry Polypeptides

In yet another aspect, the present invention provides methods for producing a transgenic plant which expresses a nucleic acid segment encoding the novel polypeptides and endotoxins of the present invention. The process of producing transgenic plants is well-known in the art. In general, the method comprises transforming a suitable host cell with a DNA segment which contains a promoter operatively linked to a coding region that encodes one or more CryET31, CryET40, CryET43, CryET44, CryET45, CryET46, CryET47, CryET49, CryET51, CryET52, CryET53, CryET54, CryET55, CryET56, CryET57, CryET59, CryET60, CryET61, CryET62, CryET63, CryET64, CryET66, CryET67, CryET68, CryET72, CryET73, and CryET83 polypeptides. Such a coding region is generally operatively linked to a transcription-terminating region, whereby the promoter is capable of driving the transcription of the coding region in the cell, and hence providing the cell the ability to produce the polypeptide *in vivo*. Alternatively, in instances where it is desirable to control, regulate, or decrease the amount of a particular recombinant crystal protein expressed in a particular transgenic cell, the invention also provides for the expression of crystal protein antisense mRNA. The use of antisense mRNA as a means of controlling or decreasing the amount of a given protein of interest in a cell is well-known in the art.

Another aspect of the invention comprises transgenic plants which express a gene or gene segment encoding one or more of the novel polypeptide compositions disclosed herein. As used herein, the term "transgenic plant" is intended to refer to a plant that has incorporated DNA sequences, including but not limited to genes which are perhaps not normally present, DNA sequences not normally transcribed into RNA or translated into a protein ("expressed"), or any other genes or DNA sequences which one desires to introduce into the non-transformed plant, such as genes which may normally be present in the non-transformed plant but which one desires to either genetically engineer or to have altered expression.

It is contemplated that in some instances either the nuclear or plastidic genome, or both, of a transgenic plant of the present invention will have been augmented through the stable

introduction of one or more *cryET31, cryET40, cryET43, cryET44, cryET45, cryET46, cryET47, cryET49, cryET51, cryET52, cryET53, cryET54, cryET55, cryET56, cryET57, cryET59, cryET60, cryET61, cryET62, cryET63, cryET64, cryET66, cryET67, cryET68, cryET72, cryET73,* and *cryET83* transgenes, either native, synthetically modified, or mutated. In some instances, more than one transgene will be incorporated into one or more genomes of the transformed host plant cell. Such is the case when more than one crystal protein-encoding DNA segment is incorporated into the genome of such a plant. In certain situations, it may be desirable to have one, two, three, four, or even more *B. thuringiensis* crystal proteins (either native or recombinantly-engineered) incorporated and stably expressed in the transformed transgenic plant.

A preferred gene which may be introduced includes, for example, a crystal protein-encoding DNA sequence from bacterial origin, and particularly one or more of those described herein which are obtained from *Bacillus* spp. Highly preferred nucleic acid sequences are those obtained from *B. thuringiensis*, or any of those sequences which have been genetically engineered to decrease or increase the insecticidal activity of the crystal protein in such a transformed host cell.

Means for transforming a plant cell and the preparation of a transgenic cell line are well-known in the art, and are discussed herein. Vectors, plasmids, cosmids, YACs (yeast artificial chromosomes) and DNA segments for use in transforming such cells will, of course, generally comprise either the operons, genes, or gene-derived sequences of the present invention, either native, or synthetically-derived, and particularly those encoding the disclosed crystal proteins. These DNA constructs can further include structures such as promoters, enhancers, polylinkers, or even gene sequences which have positively- or negatively-regulating activity upon the particular genes of interest as desired. The DNA segment or gene may encode either a native or modified crystal protein, which will be expressed in the resultant recombinant cells, and/or which will impart an improved phenotype to the regenerated plant.

Such transgenic plants may be desirable for increasing the insecticidal resistance of a monocotyledonous or dicotyledonous plant, by incorporating into such a plant, a transgenic DNA segment encoding one or more CryET31, CryET40, CryET43, CryET44, CryET45, CryET46, CryET47, CryET49, CryET51, CryET52, CryET53, CryET54, CryET55, CryET56, CryET57, CryET59, CryET60, CryET61, CryET62, CryET63, CryET64, CryET66, CryET67,

CryET68, CryET72, CryET73, and CryET83 polypeptides which are toxic to a lepidopteran insect. Particularly preferred plants include turf grasses, kapok, sorghum, cotton, corn, soybeans, oats, rye, wheat, flax, tobacco, rice, tomatoes, potatoes, or other vegetables, ornamental plants, fruit trees, and the like.

In a related aspect, the present invention also encompasses a seed produced by the transformed plant, a progeny from such seed, and a seed produced by the progeny of the original transgenic plant, produced in accordance with the above process. Such progeny and seeds will have a crystal protein-encoding transgene stably incorporated into their genome, and such progeny plants will inherit the traits afforded by the introduction of a stable transgene in Mendelian fashion. All such transgenic plants having incorporated into their genome transgenic DNA segments encoding one or more CryET31, CryET40, CryET43, CryET44, CryET45, CryET46, CryET47, CryET49, CryET51, CryET52, CryET53, CryET54, CryET55, CryET56, CryET57, CryET59, CryET60, CryET61, CryET62, CryET63, CryET64, CryET66, CryET67, CryET68, CryET72, CryET73, and CryET83 crystal proteins or polypeptides are aspects of this invention. As well-known to those of skill in the art, a progeny of a plant is understood to mean any offspring or any descendant from such a plant, but in this case means any offspring or any descendant which also contains the transgene.

2.5 Site-Specific Mutagenesis

Site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent proteins or peptides, through specific mutagenesis of the underlying DNA. The technique further provides a ready ability to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA. The technique of site-specific mutagenesis is well known in the art, as exemplified by various publications.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of a double stranded vector which includes within its sequence a DNA sequence which encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original

non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement.

The preparation of sequence variants of the endotoxin-encoding nucleic acid segments using site-directed mutagenesis is provided as a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants.

2.6 Antibody Compositions and Methods of Making

In particular embodiments, the inventors contemplate the use of antibodies, either monoclonal (mAbs) or polyclonal which bind to one or more of the polypeptides disclosed herein. Means for preparing and characterizing antibodies are well known in the art (See, *e.g.*, Harlow and Lane, 1988; incorporated herein by reference). mAbs may be readily prepared through use of well-known techniques, such as those exemplified in U. S. Patent 4,196,265, incorporated herein by reference.

2.7 ELISAs and Immunoprecipitation

ELISAs may be used in conjunction with the invention. Many different protocols exist for performing ELISAs. These are well known to those of ordinary skill in the art. Examples of basic ELISA protocols may be found in any standard molecular biology laboratory manual (e.g. Sambrook, Fritsch, and Maniatis, eds. Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989).

2.8 Western Blots

The compositions of the present invention will find great use in immunoblot or western blot analysis. Methods of performing immunoblot and western blot analysis are well known to those of skill in the are (see Sambrook, et al, ibid). Immunologically-based detection methods for use in conjunction with Western blotting include enzymatically-, radiolabel-, or fluorescently-tagged secondary antibodies against the toxin moiety are considered to be of particular use in this regard.

2.9 Crystal Protein Screening and Detection Kits

The present invention contemplates methods and kits for screening samples suspected of containing crystal protein polypeptides or crystal protein-related polypeptides, or cells producing such polypeptides. A kit may contain one or more antibodies of the present invention, and may also contain reagent(s) for detecting an interaction between a sample and an antibody of the present invention. The provided reagent(s) can be radio-, fluorescently- or enzymatically-labeled or even epitope or ligand tagged. The kit can contain a known radiolabeled agent capable of binding or interacting with a nucleic acid or antibody of the present invention.

The reagent(s) of the kit can be provided as a liquid solution, attached to a solid support or as a dried powder. Preferably, when the reagent(s) are provided in a liquid solution, the liquid solution is an aqueous solution. Preferably, when the reagent(s) provided are attached to a solid support, the solid support can be chromatograph media, a test plate having a plurality of wells, or a microscope slide. When the reagent(s) provided are a dry powder, the powder can be reconstituted by the addition of a suitable solvent, that may be provided.

In still further embodiments, the present invention concerns immunodetection methods and associated kits. It is proposed that the crystal proteins or peptides of the present invention may be employed to detect antibodies having reactivity therewith, or, alternatively, antibodies prepared in accordance with the present invention, may be employed to detect crystal proteins or crystal protein-related epitope-containing peptides. In general, these methods will include first obtaining a sample suspected of containing such a protein, peptide or antibody, contacting the sample with an antibody or peptide in accordance with the present invention, as the case may be, under conditions effective to allow the formation of an immunocomplex, and then detecting the presence of the immunocomplex.

In general, the detection of immunocomplex formation is quite well known in the art and may be achieved through the application of numerous approaches. For example, the present invention contemplates the application of ELISA, RIA, immunoblot (*e.g.*, dot blot), indirect immunofluorescence techniques and the like. One may find additional advantages through the use of a secondary binding ligand such as a second antibody or a biotin/avidin ligand binding arrangement, as is known in the art.

For assaying purposes, it is proposed that virtually any sample suspected of comprising either a crystal protein or peptide or a crystal protein-related peptide or antibody sought to be

detected, as the case may be, may be employed. It is contemplated that such embodiments may have application in the titering of antigen or antibody samples, in the selection of hybridomas, and the like. In related embodiments, the present invention contemplates the preparation of kits that may be employed to detect the presence of crystal proteins or related peptides and/or antibodies in a sample. Samples may include cells, cell supernatants, cell suspensions, cell extracts, enzyme fractions, protein extracts, or other cell-free compositions suspected of containing crystal proteins or peptides.

Generally speaking, kits in accordance with the present invention will include a suitable crystal protein, peptide or an antibody directed against such a protein or peptide, together with an immunodetection reagent and a means for containing the antibody or antigen and reagent. The immunodetection reagent will typically comprise a label associated with the antibody or antigen, or associated with a secondary binding ligand. Exemplary ligands might include a secondary antibody directed against the first antibody or antigen or a biotin or avidin (or streptavidin) ligand having an associated label. Of course, as noted above, a number of exemplary labels are known in the art and all such labels may be employed in connection with the present invention.

The container will generally include a vial into which the antibody, antigen or detection reagent may be placed, and preferably suitably aliquotted. The kits of the present invention will also typically include a means for containing the antibody, antigen, and reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

2.10 Epitopic Core Sequences

The present invention is also directed to protein or peptide compositions, free from total cells and other peptides, which comprise a purified protein or peptide which incorporates an epitope that is immunologically cross-reactive with one or more anti-crystal protein antibodies. In particular, the invention concerns epitopic core sequences derived from Cry proteins or peptides.

As used herein, the term "incorporating an epitope(s) that is immunologically cross-reactive with one or more anti-crystal protein antibodies" is intended to refer to a peptide or protein antigen which includes a primary, secondary or tertiary structure similar to an epitope located within a crystal protein or polypeptide. The level of similarity will generally be to such a degree that monoclonal or polyclonal antibodies directed against the crystal protein or

polypeptide will also bind to, react with, or otherwise recognize, the cross-reactive peptide or protein antigen. Various immunoassay methods may be employed in conjunction with such antibodies, such as, for example, Western blotting, ELISA, RIA, and the like, all of which are known to those of skill in the art. The identification of Cry immunodominant epitopes, and/or their functional equivalents, suitable for use in vaccines is a relatively straightforward matter (e.g. U. S. Patent 4,554,101; Jameson and Wolf, 1988; Wolf *et al.*, 1988; U. S. Patent 4,554,101). The amino acid sequence of these "epitopic core sequences" may then be readily incorporated into peptides, either through the application of peptide synthesis or recombinant technology.

Preferred peptides for use in accordance with the present invention will generally be on the order of about 8 to about 20 amino acids in length, and more preferably about 8 to about 15 amino acids in length. It is proposed that particular advantages of the present invention may be realized through the preparation of synthetic peptides which include modified and/or extended epitopic/immunogenic core sequences which result in a "universal" epitopic peptide directed to crystal proteins, and in particular CryET31, CryET40, CryET43, CryET44, CryET45, CryET46, CryET47, CryET49, CryET51, CryET52, CryET53, CryET54, CryET55, CryET56, CryET57, CryET59, CryET60, CryET61, CryET62, CryET63, CryET64, CryET66, CryET67, CryET68, CryET72, CryET73, CryET83 and related sequences. These epitopic core sequences are identified herein in particular aspects as hydrophilic regions of the particular polypeptide antigen.

Computerized peptide sequence analysis programs (*e.g.*, DNAStar® software, DNAStar, Inc., Madison, WI) may also be useful in designing synthetic peptides in accordance with the present disclosure.

Syntheses of epitopic sequences, or peptides which include an antigenic epitope within their sequence, are readily achieved using conventional synthetic techniques such as the solid phase method (*e.g.*, through the use of commercially available peptide synthesizer such as an Applied Biosystems Model 430A Peptide Synthesizer).

2.11 Biological Functional Equivalents

Modification and changes may be made in the structure of the peptides of the present invention and DNA segments which encode them and still obtain a functional molecule that encodes a protein or peptide with desirable characteristics. The following is a discussion based

upon changing the amino acids of a protein to create an equivalent, or even an improved, second-generation molecule. In particular embodiments of the invention, mutated crystal proteins are contemplated to be useful for increasing the insecticidal activity of the protein, and consequently increasing the insecticidal activity and/or expression of the recombinant transgene in a plant cell. The amino acid changes may be achieved by changing the codons of the DNA sequence, according to the codons given in Table 1.

TABLE 1

Amino Acids			Codons					
Alanine	Ala	A	GCA	GCC	GCG	GCU		
Cysteine	Cys	C	UGC	UGU				
Aspartic acid	Asp	D	GAC	GAU				
Glutamic acid	Glu	E	GAA	GAG				
Phenylalanine	Phe	F	UUC	UUU				
Glycine	Gly	G	GGA	GGC	GGG	GGU		
Histidine	His	H	CAC	CAU				
Isoleucine	Ile	I	AUA	AUC	AUU			
Lysine	Lys	K	AAA	AAG				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	M	AUG					
Asparagine	Asn	N	AAC	AAU				
Proline	Pro	P	CCA	CCC	CCG	CCU		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU		
Valine	Val	V	GUA	GUC	GUG	GUU		
Tryptophan	Trp	W	UGG					
Tyrosine	Tyr	Y	UAC	UAU				

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated by the inventors that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporate herein by reference). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics (Kyte and Doolittle, 1982), these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (–0.4); threonine (–0.7); serine (–0.8); tryptophan (–0.9); tyrosine (–1.3); proline (–1.6); histidine (–3.2); glutamate (–3.5); glutamine (–3.5); aspartate (–3.5); asparagine (–3.5); lysine (–3.9); and arginine (–4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.*, still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U. S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U. S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (–0.4); proline (–0.5 ± 1); alanine (–0.5); histidine (–0.5); cysteine (–1.0); methionine (–1.3); valine (–1.5); leucine (–1.8); isoleucine (–1.8); tyrosine (–2.3); phenylalanine (–2.5); tryptophan (–3.4).

It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose

hydrophilicity values are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

2.12 Insecticidal Compositions and Methods of Use

The inventors contemplate that the crystal protein compositions disclosed herein will find particular utility as insecticides for topical and/or systemic application to field crops, grasses, fruits and vegetables, and ornamental plants. In a preferred embodiment, the bioinsecticide composition comprises an oil flowable suspension of bacterial cells which expresses a novel crystal protein disclosed herein. Preferably the cells are *B. thuringiensis* NRRL B-21921, NRRL B-21922, NRRL B-21923, NRRL B-21924, NRRL B-21925, NRRL B-21926, NRRL B-21927, NRRL B-21928, NRRL B-21929, NRRL B-21930, NRRL B-21931, NRRL B-21932, NRRL B-21933, NRRL B-21934, NRRL B-21935, NRRL B-21936, NRRL B-21937, NRRL B-21938, NRRL B-21939, NRRL B-21940, NRRL B-21941, NRRL B-21942, NRRL B-21943, and NRRL B-21944, however, any such bacterial host cell expressing the novel nucleic acid segments disclosed herein and producing a crystal protein is contemplated to be useful, such as *B. thuringiensis*, *B. megaterium, B. subtilis, E. coli,* or *Pseudomonas* spp.

In another important embodiment, the bioinsecticide composition comprises a water dispersible granule. This granule comprises bacterial cells which expresses a novel crystal protein disclosed herein. Preferred bacterial cells are *B. thuringiensis* NRRL B-21921, NRRL B-21922, NRRL B-21923, NRRL B-21924, NRRL B-21925, NRRL B-21926, NRRL B-21927, NRRL B-21928, NRRL B-21929, NRRL B-21930, NRRL B-21931, NRRL B-21932, NRRL B-21933, NRRL B-21934, NRRL B-21935, NRRL B-21936, NRRL B-21937, NRRL B-21938, NRRL B-21939, NRRL B-21940, NRRL B-21941, NRRL B-21942, NRRL B-21943, and NRRL B-21944, however, bacteria such as *B. thuringiensis, B. megaterium, B. subtilis, E. coli*, or

Pseudomonas spp. cells transformed with a DNA segment disclosed herein and expressing the crystal protein are also contemplated to be useful.

In a third important embodiment, the bioinsecticide composition comprises a wettable powder, dust, pellet, or collodial concentrate. This powder comprises bacterial cells which expresses a novel crystal protein disclosed herein. Preferred bacterial cells are *B. thuringiensis* NRRL B-21921, NRRL B-21922, NRRL B-21923, NRRL B-21924, NRRL B-21925, NRRL B-21926, NRRL B-21927, NRRL B-21928, NRRL B-21929, NRRL B-21930, NRRL B-21931, NRRL B-21932, NRRL B-21933, NRRL B-21934, NRRL B-21935, NRRL B-21936, NRRL B-21937, NRRL B-21938, NRRL B-21939, NRRL B-21940, NRRL B-21941, NRRL B-21942, NRRL B-21943, and NRRL B-21944 cells, however, bacteria such as *B. thuringiensis*, *B. megaterium*, *B. subtilis*, *E. coli*, or *Pseudomonas* spp. cells transformed with a DNA segment disclosed herein and expressing the crystal protein are also contemplated to be useful. Such dry forms of the insecticidal compositions may be formulated to dissolve immediately upon wetting, or alternatively, dissolve in a controlled-release, sustained-release, or other time-dependent manner.

In a fourth important embodiment, the bioinsecticide composition comprises an aqueous suspension of bacterial cells such as those described above which express the crystal protein. Such aqueous suspensions may be provided as a concentrated stock solution which is diluted prior to application, or alternatively, as a diluted solution ready-to-apply.

For these methods involving application of bacterial cells, the cellular host containing the crystal protein gene(s) may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the *B. thuringiensis* gene. These cells may then be harvested in accordance with conventional ways. Alternatively, the cells can be treated prior to harvesting.

When the insecticidal compositions comprise intact *B. thuringiensis* cells expressing the protein of interest, such bacteria may be formulated in a variety of ways. They may be employed as wettable powders, granules or dusts, by mixing with various diluents, inert materials, such as inorganic minerals (phyllosilicates, carbonates, sulfates, phosphates, and the like) or botanical materials (powdered corncobs, rice hulls, walnut shells, and the like). The formulations may include spreader-sticker adjuvants, stabilizing agents, other pesticidal additives, or surfactants. Liquid formulations may be aqueous-based or non-aqueous and employed as foams, suspensions,

emulsifiable concentrates, or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants, or polymers.

Alternatively, the novel insecticidal polypeptides may be prepared by native or recombinant bacterial expression systems *in vitro* and isolated for subsequent field application. Such protein may be either in crude cell lysates, suspensions, colloids, *etc.*, or alternatively may be purified, refined, buffered, and/or further processed, before formulating in an active biocidal formulation. Likewise, under certain circumstances, it may be desirable to isolate crystals and/or spores from bacterial cultures expressing the crystal protein and apply solutions, suspensions, or collodial preparations of such crystals and/or spores as the active bioinsecticidal composition.

Regardless of the method of application, the amount of the active component(s) is applied at an insecticidally-effective amount, which will vary depending on such factors as, for example, the specific coleopteran insects to be controlled, the specific plant or crop to be treated, the environmental conditions, and the method, rate, and quantity of application of the insecticidally-active composition.

The insecticide compositions described may be made by formulating either the bacterial cell, crystal and/or spore suspension, or isolated protein component with the desired agriculturally-acceptable carrier. The compositions may be formulated prior to administration in an appropriate means such as lyophilized, freeze-dried, dessicated, or in an aqueous carrier, medium or suitable diluent, such as saline or other buffer. The formulated compositions may be in the form of a dust or granular material, or a suspension in oil (vegetable or mineral), or water or oil/water emulsions, or as a wettable powder, or in combination with any other carrier material suitable for agricultural application. Suitable agricultural carriers can be solid or liquid and are well known in the art. The term "agriculturally-acceptable carrier" covers all adjuvants, *E. coli*, inert components, dispersants, surfactants, tackifiers, binders, *etc.* that are ordinarily used in insecticide formulation technology; these are well known to those skilled in insecticide formulation. The formulations may be mixed with one or more solid or liquid adjuvants and prepared by various means, *E. coli*, by homogeneously mixing, blending and/or grinding the insecticidal composition with suitable adjuvants using conventional formulation techniques.

The insecticidal compositions of this invention are applied to the environment of the target lepidopteran insect, typically onto the foliage of the plant or crop to be protected, by conventional methods, preferably by spraying. The strength and duration of insecticidal

application will be set with regard to conditions specific to the particular pest(s), crop(s) to be treated and particular environmental conditions. The proportional ratio of active ingredient to carrier will naturally depend on the chemical nature, solubility, and stability of the insecticidal composition, as well as the particular formulation contemplated.

Other application techniques, including dusting, sprinkling, soaking, soil injection, seed coating, seedling coating, spraying, aerating, misting, atomizing, and the like, are also feasible and may be required under certain circumstances such as *e.g.,* insects that cause root or stalk infestation, or for application to delicate vegetation or ornamental plants. These application procedures are also well-known to those of skill in the art.

The insecticidal composition of the invention may be employed in the method of the invention singly or in combination with other compounds, including and not limited to other pesticides. The method of the invention may also be used in conjunction with other treatments such as surfactants, detergents, polymers or time-release formulations. The insecticidal compositions of the present invention may be formulated for either systemic or topical use.

The concentration of insecticidal composition which is used for environmental, systemic, or foliar application will vary widely depending upon the nature of the particular formulation, means of application, environmental conditions, and degree of biocidal activity. Typically, the bioinsecticidal composition will be present in the applied formulation at a concentration of at least about 1% by weight and may be up to and including about 99% by weight. Dry formulations of the polypeptide compositions may be from about 1% to about 99% or more by weight of the protein composition, while liquid formulations may generally comprise from about 1% to about 99% or more of the active ingredient by weight. Formulations which comprise intact bacterial cells will generally contain from about 10^4 to about 10^7 cells/mg.

The insecticidal formulation may be administered to a particular plant or target area in one or more applications as needed, with a typical field application rate per hectare ranging on the order of from about 50 g to about 500 g of active ingredient, or of from about 500 g to about 1000 g, or of from about 1000 g to about 5000 g or more of active ingredient.

5.0 Description of Illustrative Embodiments

5.1 Some Advantages of the Invention

The use of *B. thuringiensis* insecticidal crystal protein genes for *in planta* production of insecticidal proteins, thereby conferring insect resistance on important agronomic plants, is rapidly gaining commercial acceptance in the United States and abroad. The need for new insecticidal traits does not diminish, however, with the successful deployment of a handful of *cry* genes in plants. Concerns over the potential for insect resistance development, for instance, makes it imperative that an arsenal of insecticidal proteins (i.e. *cry* genes) be assembled to provide the genetic material necessary for tomorrow's insecticidal traits. In addition, transgenic plants producing a *B. thuringiensis* Cry protein may still be susceptible to damage from secondary insect pests, thus prompting the search for additional Cry proteins with improved efficacy towards these pests. The *B. thuringiensis* crystal proteins of the present invention represent a diverse collection of insecticidal proteins, including several that are toxic towards a lepidopteran colony exhibiting resistance to certain types of Cry1 proteins. Bioassays against a wide range of lepidopteran pests confirm that these proteins possess insecticidal activity and, furthermore, that these proteins vary in efficacy against this array of target insects. This variation in the spectrum of insects affected by the toxin proteins suggests differing modes of action that may be important for future insect resistance management strategies. *In planta* expression of the *cry* genes of the present invention can confer insect resistance to the host plant as has been demonstrated for other *cry* genes from *B. thuringiensis*.

5.2 Probes and Primers

In another aspect, DNA sequence information provided by the invention allows for the preparation of relatively short DNA (or RNA) sequences having the ability to specifically hybridize to gene sequences of the selected polynucleotides disclosed herein. In these aspects, nucleic acid probes of an appropriate length are prepared based on a consideration of a selected crystal protein gene sequence, *e.g.*, a sequence such as that shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID

NO:49 and SEQ ID NO:62. The ability of such DNAs and nucleic acid probes to specifically hybridize to a crystal protein-encoding gene sequence lends them particular utility in a variety of embodiments. Most importantly, the probes may be used in a variety of assays for detecting the presence of complementary sequences in a given sample.

In certain embodiments, it is advantageous to use oligonucleotide primers. The sequence of such primers is designed using a polynucleotide of the present invention for use in detecting, amplifying or mutating a defined segment of a crystal protein gene from *B. thuringiensis* using PCR™ technology. Segments of related crystal protein genes from other species may also be amplified by PCR™ using such primers.

To provide certain of the advantages in accordance with the present invention, a preferred nucleic acid sequence employed for hybridization studies or assays includes sequences that are complementary to at least a 14 to 30 or so long nucleotide stretch of a crystal protein-encoding sequence, such as that shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49 and SEQ ID NO:62. A size of at least about 14 or so nucleotides in length helps to ensure that the fragment will be of sufficient length to form a duplex molecule that is both stable and selective. Molecules having complementary sequences over stretches greater than about 14 or so bases in length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of about 14 to about 20 or so nucleotides, or even longer where desired. Such fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the PCR™ technology of U. S. Patents 4,683,195, and 4,683,202, herein incorporated by reference, or by excising selected DNA fragments from recombinant plasmids containing appropriate inserts and suitable restriction sites.

5.3 Expression Vectors

The present invention contemplates an expression vector comprising a polynucleotide of the present invention. Thus, in one embodiment an expression vector is an isolated and purified

DNA molecule comprising a promoter operatively linked to an coding region that encodes a polypeptide of the present invention, which coding region is operatively linked to a transcription-terminating region, whereby the promoter drives the transcription of the coding region.

As used herein, the term "operatively linked" means that a promoter is connected to an coding region in such a way that the transcription of that coding region is controlled and regulated by that promoter. Means for operatively linking a promoter to a coding region are well known in the art.

In a preferred embodiment, the recombinant expression of DNAs encoding the crystal proteins of the present invention is preferable in a *Bacillus* host cell. Preferred host cells include *B. thuringiensis*, *B. megaterium*, *B. subtilis*, and related bacilli, with *B. thuringiensis* host cells being highly preferred. Promoters that function in bacteria are well-known in the art. An exemplary and preferred promoter for the *Bacillus* crystal proteins include any of the known crystal protein gene promoters, including the *cryET31*, *cryET40*, *cryET43*, *cryET44*, *cryET45*, *cryET46*, *cryET47*, *cryET49*, *cryET51*, *cryET52*, *cryET53*, *cryET54*, *cryET55*, *cryET56*, *cryET57*, *cryET59*, *cryET60*, *cryET61*, *cryET62*, *cryET63*, *cryET64*, *cryET66*, *cryET67*, *cryET68*, *cryET72*, *cryET73*, and *cryET83* gene promoters. Alternatively, mutagenized or recombinant crystal protein-encoding gene promoters may be engineered by the hand of man and used to promote expression of the novel gene segments disclosed herein.

In an alternate embodiment, the recombinant expression of DNAs encoding the crystal proteins of the present invention is performed using a transformed Gram-negative bacterium such as an *E. coli* or *Pseudomonas* spp. host cell. Promoters which function in high-level expression of target polypeptides in *E. coli* and other Gram-negative host cells are also well-known in the art.

Where an expression vector of the present invention is to be used to transform a plant, a promoter is selected that has the ability to drive expression in plants. Promoters that function in plants are also well known in the art. Useful in expressing the polypeptide in plants are promoters that are inducible, viral, synthetic, constitutive as described (Poszkowski *et al.*, 1989; Odell *et al.*, 1985), and temporally regulated, spatially regulated, and spatio-temporally regulated (Chau *et al.*, 1989).

A promoter is also selected for its ability to direct the transformed plant cell's or transgenic plant's transcriptional activity to the coding region. Structural genes can be driven by

a variety of promoters in plant tissues. Promoters can be near-constitutive, such as the CaMV 35S promoter, or tissue-specific or developmentally specific promoters affecting dicots or monocots.

Where the promoter is a near-constitutive promoter such as CaMV 35S, increases in polypeptide expression are found in a variety of transformed plant tissues (*e.g.*, callus, leaf, seed and root). Alternatively, the effects of transformation can be directed to specific plant tissues by using plant integrating vectors containing a tissue-specific promoter.

An exemplary tissue-specific promoter is the lectin promoter, which is specific for seed tissue. The Lectin protein in soybean seeds is encoded by a single gene (*Le1*) that is only expressed during seed maturation and accounts for about 2 to about 5% of total seed mRNA. The lectin gene and seed-specific promoter have been fully characterized and used to direct seed specific expression in transgenic tobacco plants (Vodkin *et al.*, 1983; Lindstrom *et al.*, 1990.)

An expression vector containing a coding region that encodes a polypeptide of interest is engineered to be under control of the lectin promoter and that vector is introduced into plants using, for example, a protoplast transformation method (Dhir *et al.*, 1991). The expression of the polypeptide is directed specifically to the seeds of the transgenic plant.

A transgenic plant of the present invention produced from a plant cell transformed with a tissue specific promoter can be crossed with a second transgenic plant developed from a plant cell transformed with a different tissue specific promoter to produce a hybrid transgenic plant that shows the effects of transformation in more than one specific tissue.

Exemplary tissue-specific promoters are corn sucrose synthetase 1 (Yang *et al.*, 1990), corn alcohol dehydrogenase 1 (Vogel *et al.*, 1989), corn light harvesting complex (Simpson, 1986), corn heat shock protein (Odell *et al.*, 1985), pea small subunit RuBP carboxylase (Poulsen *et al.*, 1986; Cashmore *et al.*, 1983), Ti plasmid mannopine synthase (Langridge *et al.*, 1989), Ti plasmid nopaline synthase (Langridge *et al.*, 1989), petunia chalcone isomerase (Van Tunen *et al.*, 1988), bean glycine rich protein 1 (Keller *et al.*, 1989), CaMV 35s transcript (Odell *et al.*, 1985) and Potato patatin (Wenzler *et al.*, 1989). Preferred promoters include a cauliflower mosaic virus (CaMV 35S) promoter, a S-E9 small subunit RuBP carboxylase promoter, a rice actin promoter, a maize histone promoter, a fused CaMV 35S-*Arabidopsis* histone promoter, a CaMV 35S promoter, a CaMV 19S promoter, a *nos* promoter, an Adh promoter, an actin promoter, a histone promoter, a ribulose bisphosphate carboxylase promoter, an R-allele

promoter, a root cell promoter, an α-tubulin promoter, an ABA-inducible promoter, a turgor-inducible promoter, a *rbcS* promoter, a corn sucrose synthetase 1 promoter, a corn alcohol dehydrogenase 1 promoter, a corn light harvesting complex promoter, a corn heat shock protein promoter, a pea small subunit RuBP carboxylase promoter, a Ti plasmid mannopine synthase promoter, a Ti plasmid nopaline synthase promoter, a petunia chalcone isomerase promoter, a bean glycine rich protein 1 promoter, a CaMV 35s transcript promoter, a potato patatin promoter, a *cab* promoter, a PEP-Carboxylase promoter and an S-E9 small subunit RuBP carboxylase promoter.

The choice of which expression vector and ultimately to which promoter a polypeptide coding region is operatively linked depends directly on the functional properties desired, *e.g.*, the location and timing of protein expression, and the host cell to be transformed. These are well known limitations inherent in the art of constructing recombinant DNA molecules. However, a vector useful in practicing the present invention is capable of directing the expression of the polypeptide coding region to which it is operatively linked.

Typical vectors useful for expression of genes in higher plants are well known in the art and include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* described (Rogers *et al.*, 1987). However, several other plant integrating vector systems are known to function in plants including pCaMVCN transfer control vector described (Fromm *et al.*, 1985). Plasmid pCaMVCN (available from Pharmacia, Piscataway, NJ) includes the cauliflower mosaic virus CaMV 35S promoter.

In preferred embodiments, the vector used to express the polypeptide includes a selection marker that is effective in a plant cell, preferably a drug resistance selection marker. One preferred drug resistance marker is the gene whose expression results in kanamycin resistance; *i.e.*, the chimeric gene containing the nopaline synthase promoter, Tn5 neomycin phosphotransferase II (*nptII*) and nopaline synthase 3′ non-translated region described (Rogers *et al.*, 1988).

RNA polymerase transcribes a coding DNA sequence through a site where polyadenylation occurs. Typically, DNA sequences located a few hundred base pairs downstream of the polyadenylation site serve to terminate transcription. Those DNA sequences are referred to herein as transcription-termination regions. Those regions are required for efficient polyadenylation of transcribed messenger RNA (mRNA).

Means for preparing expression vectors are well known in the art. Expression (transformation vectors) used to transform plants and methods of making those vectors are described in U. S. Patents 4,971,908, 4,940,835, 4,769,061 and 4,757,011, the disclosures of which are incorporated herein by reference. Those vectors can be modified to include a coding sequence in accordance with the present invention.

A variety of methods has been developed to operatively link DNA to vectors via complementary cohesive termini or blunt ends. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted and to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

A coding region that encodes a polypeptide having the ability to confer insecticidal activity to a cell is preferably a CryET31, CryET40, CryET43, CryET44, CryET45, CryET46, CryET47, CryET49, CryET51, CryET52, CryET53, CryET54, CryET55, CryET56, CryET57, CryET59, CryET60, CryET61, CryET62, CryET63, CryET64, CryET66, CryET67, CryET68, CryET72, CryET73, and CryET83 polypeptide-encoding gene.

5.7 Nomenclature of the Novel Polypeptides

The inventors have arbitrarily assigned the designation CryET31, CryET40, CryET43, CryET44, CryET45, CryET46, CryET47, CryET49, CryET51, CryET52, CryET53, CryET54, CryET56, CryET57, CryET59, CryET60, CryET61, CryET62, CryET63, CryET64, CryET66, CryET67, CryET68, CryET72, CryET73, and CryET83 to the polypeptides of this invention. Likewise, the arbitrary designations of *cryET31, cryET40, cryET43, cryET44, cryET45, cryET46, cryET47, cryET49, cryET51, cryET52, cryET53, cryET54, cryET56, cryET57, cryET59, cryET60, cryET61, cryET62, cryET63, cryET64, cryET66, cryET67, cryET68, cryET72, cryET73,* and *cryET83* have been assigned to the novel nucleic acid sequence which encodes these polypeptides, respectively. Formal assignment of gene and protein designations based on the revised nomenclature of crystal protein endotoxins will be assigned by a committee on the nomenclature of *B. thuringiensis*, formed to systematically classify *B. thuringiensis* crystal proteins. The inventors contemplate that the arbitrarily assigned designations of the present invention will be superceded by the official nomenclature assigned to these sequences.

5.8 Transformed Host Cells and Transgenic Plants

Methods and compositions for transforming a bacterium, a yeast cell, a plant cell, or an entire plant with one or more expression vectors comprising a crystal protein-encoding gene segment are further aspects of this disclosure. A transgenic bacterium, yeast cell, plant cell or plant derived from such a transformation process or the progeny and seeds from such a transgenic plant are also further embodiments of the invention.

Means for transforming bacteria and yeast cells are well known in the art. Typically, means of transformation are similar to those well known means used to transform other bacteria or yeast such as *E. coli* or *Saccharomyces cerevisiae*. Methods for DNA transformation of plant cells include *Agrobacterium*-mediated plant transformation, protoplast transformation, gene transfer into pollen, injection into reproductive organs, injection into immature embryos and particle bombardment. Each of these methods has distinct advantages and disadvantages. Thus, one particular method of introducing genes into a particular plant strain may not necessarily be the most effective for another plant strain, but it is well known which methods are useful for a particular plant strain.

There are many methods for introducing transforming DNA segments into cells, but not all are suitable for delivering DNA to plant cells. Suitable methods are believed to include virtually any method by which DNA can be introduced into a cell, such as by *Agrobacterium* infection, direct delivery of DNA such as, for example, by PEG-mediated transformation of protoplasts (Omirulleh *et al.*, 1993), by desiccation/inhibition-mediated DNA uptake, by electroporation, by agitation with silicon carbide fibers, by acceleration of DNA coated particles, *etc.* In certain embodiments, acceleration methods are preferred and include, for example, microprojectile bombardment and the like.

Technology for introduction of DNA into cells is well-known to those of skill in the art. Four general methods for delivering a gene into cells have been described: (1) chemical methods (Graham and van der Eb, 1973; Zatloukal *et al.*, 1992); (2) physical methods such as microinjection (Capecchi, 1980), electroporation (Wong and Neumann, 1982; Fromm *et al.*, 1985; U. S. Patent No. 5,384,253) and the gene gun (Johnston and Tang, 1994; Fynan *et al.*, 1993); (3) viral vectors (Clapp, 1993; Lu *et al.*, 1993; Eglitis and Anderson, 1988a; 1988b); and (4) receptor-mediated mechanisms (Curiel *et al.*, 1991; 1992; Wagner *et al.*, 1992).

5.8.3 *Agrobacterium*-Mediated Transfer

Agrobacterium-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art. See, for example, the methods described (Fraley *et al.*, 1985; Rogers *et al.*, 1987). Further, the integration of the Ti-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences, and intervening DNA is usually inserted into the plant genome as described (Spielmann *et al.*, 1986; Jorgensen *et al.*, 1987).

Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations as described (Klee *et al.*, 1985). Moreover, recent technological advances in vectors for *Agrobacterium*-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate construction of vectors capable of expressing various polypeptide coding genes. The vectors described (Rogers *et al.*, 1987), have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes and are suitable for present purposes. In addition, *Agrobacterium* containing both armed and disarmed Ti genes can be used for the transformations. In those plant strains where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer.

Agrobacterium-mediated transformation of leaf disks and other tissues such as cotyledons and hypocotyls appears to be limited to plants that *Agrobacterium* naturally infects. *Agrobacterium*-mediated transformation is most efficient in dicotyledonous plants. Few monocots appear to be natural hosts for *Agrobacterium*, although transgenic plants have been produced in asparagus using *Agrobacterium* vectors as described (Bytebier *et al.*, 1987). Therefore, commercially important cereal grains such as rice, corn, and wheat must usually be transformed using alternative methods. However, as mentioned above, the transformation of asparagus using *Agrobacterium* can also be achieved (see, for example, Bytebier *et al.*, 1987).

A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single gene on one chromosome. Such transgenic plants can be referred to as being

heterozygous for the added gene. However, inasmuch as use of the word "heterozygous" usually implies the presence of a complementary gene at the same locus of the second chromosome of a pair of chromosomes, and there is no such gene in a plant containing one added gene as here, it is believed that a more accurate name for such a plant is an independent segregant, because the added, exogenous gene segregates independently during mitosis and meiosis.

More preferred is a transgenic plant that is homozygous for the added structural gene; *i.e.*, a transgenic plant that contains two added genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) an independent segregant transgenic plant that contains a single added gene, germinating some of the seed produced and analyzing the resulting plants produced for enhanced carboxylase activity relative to a control (native, non-transgenic) or an independent segregant transgenic plant.

It is to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating added, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes that encode a polypeptide of interest. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated.

Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments (see, *e.g.*, Potrykus *et al.*, 1985; Lorz *et al.*, 1985; Fromm *et al.*, 1985; Uchimiya *et al.*, 1986; Callis *et al.*, 1987; Marcotte *et al.*, 1988).

Application of these systems to different plant strains depends upon the ability to regenerate that particular plant strain from protoplasts. Illustrative methods for the regeneration of cereals from protoplasts are described (Fujimura *et al.*, 1985; Toriyama *et al.*, 1986; Yamada *et al.*, 1986; Abdullah *et al.*, 1986).

5.8.4 Other Transformation Methods

Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments (see, *e.g.*, Potrykus *et al.*, 1985; Lorz *et al.*, 1985; Fromm *et al.*, 1985; Uchimiya *et al.*, 1986; Callis *et al.*, 1987; Marcotte *et al.*, 1988).

Illustrative methods for the regeneration of cereals from protoplasts are described (Fujimura *et al.*, 1985; Toriyama *et al.*, 1986; Yamada *et al.*, 1986; Abdullah *et al.*, 1986).

5.8.5 Gene Expression in Plants

Although great progress has been made in recent years with respect to preparation of transgenic plants which express bacterial proteins such as *B. thuringiensis* crystal proteins, the results of expressing native bacterial genes in plants are often disappointing. In recent years, however, several potential factors have been implicated as responsible in varying degrees for the level of protein expression from a particular coding sequence. For example, scientists now know that maintaining a significant level of a particular mRNA in the cell is indeed a critical factor. Unfortunately, the causes for low steady state levels of mRNA encoding foreign proteins are many. First, full length RNA synthesis may not occur at a high frequency. This could, for example, be caused by the premature termination of RNA during transcription or due to unexpected mRNA processing during transcription. Second, full length RNA may be produced in the plant cell, but then processed (splicing, polyA addition) in the nucleus in a fashion that creates a nonfunctional mRNA. If the RNA is not properly synthesized, terminated and polyadenylated, it cannot move to the cytoplasm for translation. Similarly, in the cytoplasm, if mRNAs have reduced half lives (which are determined by their primary or secondary sequence) inisufficient protein product will be produced. In addition, there is an effect, whose magnitude is uncertain, of translational efficiency on mRNA half-life. In addition, every RNA molecule folds into a particular structure, or perhaps family of structures, which is determined by its sequence. The particular structure of any RNA might lead to greater or lesser stability in the cytoplasm. Structure *per se* is probably also a determinant of mRNA processing in the nucleus. It is likely that dramatically changing the sequence of an RNA will have a large effect on its folded structure It is likely that structure *per se* or particular structural features also have a role in determining RNA stability.

To overcome these limitations in foreign gene expression, researchers have identified particular sequences and signals in RNAs that have the potential for having a specific effect on RNA stability. In certain embodiments of the invention, therefore, there is a desire to optimize expression of the disclosed nucleic acid segments *in planta*. One particular method of doing so, is by alteration of the bacterial gene to remove sequences or motifs which decrease expression in

a transformed plant cell. The process of engineering a coding sequence for optimal expression *in planta* is often referred to as "plantizing" a DNA sequence.

Particularly problematic sequences are those which are A+T rich. Unfortunately, since *B. thuringiensis* has an A+T rich genome, native crystal protein gene sequences must often be modified for optimal expression in a plant. The sequence motif ATTTA (or AUUUA as it appears in RNA) has been implicated as a destabilizing sequence in mammalian cell mRNA (Shaw and Kamen, 1986). Many short lived mRNAs have A+T rich 3′ untranslated regions, and these regions often have the ATTTA sequence, sometimes present in multiple copies or as multimers (*e.g.*, ATTTATTTA...). Shaw and Kamen showed that the transfer of the 3′ end of an unstable mRNA to a stable RNA (globin or VA1) decreased the stable RNA's half life dramatically. They further showed that a pentamer of ATTTA had a profound destabilizing effect on a stable message, and that this signal could exert its effect whether it was located at the 3′ end or within the coding sequence. However, the number of ATTTA sequences and/or the sequence context in which they occur also appear to be important in determining whether they function as destabilizing sequences. Shaw and Kamen showed that a trimer of ATTTA had much less effect than a pentamer on mRNA stability and a dimer or a monomer had no effect on stability (Shaw and Kamen, 1987). Note that multimers of ATTTA such as a pentamer automatically create an A+T rich region. This was shown to be a cytoplasmic effect, not nuclear. In other unstable mRNAs, the ATTTA sequence may be present in only a single copy, but it is often contained in an A+T rich region. From the animal cell data collected to date, it appears that ATTTA at least in some contexts is important in stability, but it is not yet possible to predict which occurrences of ATTTA are destabiling elements or whether any of these effects are likely to be seen in plants.

Some studies on mRNA degradation in animal cells also indicate that RNA degradation may begin in some cases with nucleolytic attack in A+T rich regions. It is not clear if these cleavages occur at ATTTA sequences. There are also examples of mRNAs that have differential stability depending on the cell type in which they are expressed or on the stage within the cell cycle at which they are expressed. For example, histone mRNAs are stable during DNA synthesis but unstable if DNA synthesis is disrupted. The 3′ end of some histone mRNAs seems to be responsible for this effect (Pandey and Marzluff, 1987). It does not appear to be mediated by ATTTA, nor is it clear what controls the differential stability of this mRNA. Another

example is the differential stability of IgG mRNA in B lymphocytes during B cell maturation (Genovese and Milcarek, 1988). A final example is the instability of a mutant β-thallesemic globin mRNA. In bone marrow cells, where this gene is normally expressed, the mutant mRNA is unstable, while the wild-type mRNA is stable. When the mutant gene is expressed in HeLa or L cells *in vitro*, the mutant mRNA shows no instability (Lim *et al.*, 1988). These examples all provide evidence that mRNA stability can be mediated by cell type or cell cycle specific factors. Furthermore this type of instability is not yet associated with specific sequences. Given these uncertainties, it is not possible to predict which RNAs are likely to be unstable in a given cell. In addition, even the ATTTA motif may act differentially depending on the nature of the cell in which the RNA is present. Shaw and Kamen (1987) have reported that activation of protein kinase C can block degradation mediated by ATTTA.

The addition of a polyadenylate string to the 3′ end is common to most eukaryotic mRNAs, both plant and animal. The currently accepted view of polyA addition is that the nascent transcript extends beyond the mature 3′ terminus. Contained within this transcript are signals for polyadenylation and proper 3′ end formation. This processing at the 3′ end involves cleavage of the mRNA and addition of polyA to the mature 3′ end. By searching for consensus sequences near the polyA tract in both plant and animal mRNAs, it has been possible to identify consensus sequences that apparently are involved in polyA addition and 3′ end cleavage. The same consensus sequences seem to be important to both of these processes. These signals are typically a variation on the sequence AATAAA. In animal cells, some variants of this sequence that are functional have been identified; in plant cells there seems to be an extended range of functional sequences (Wickens and Stephenson, 1984; Dean *et al.*, 1986). Because all of these consensus sequences are variations on AATAAA, they all are A+T rich sequences. This sequence is typically found 15 to 20 bp before the polyA tract in a mature mRNA. Studies in animal cells indicate that this sequence is involved in both polyA addition and 3′ maturation. Site directed mutations in this sequence can disrupt these functions (Conway and Wickens, 1988; Wickens *et al.*, 1987). However, it has also been observed that sequences up to 50 to 100 bp 3′ to the putative polyA signal are also required; *i.e.*, a gene that has a normal AATAAA but has been replaced or disrupted downstream does not get properly polyadenylated (Gil and Proudfoot, 1984; Sadofsky and Alwine, 1984; McDevitt *et al.*, 1984). That is, the polyA signal itself is not sufficient for complete and proper processing. It is not yet known what specific downstream

sequences are required in addition to the polyA signal, or if there is a specific sequence that has this function. Therefore, sequence analysis can only identify potential polyA signals.

In naturally occurring mRNAs that are normally polyadenylated, it has been observed that disruption of this process, either by altering the polyA signal or other sequences in the mRNA, profound effects can be obtained in the level of functional mRNA. This has been observed in several naturally occurring mRNAs, with results that are gene-specific so far.

It has been shown that in natural mRNAs proper polyadenylation is important in mRNA accumulation, and that disruption of this process can effect mRNA levels significantly. However, insufficient knowledge exists to predict the effect of changes in a normal gene. In a heterologous gene, it is even harder to predict the consequences. However, it is possible that the putative sites identified are dysfunctional. That is, these sites may not act as proper polyA sites, but instead function as aberrant sites that give rise to unstable mRNAs.

In animal cell systems, AATAAA is by far the most common signal identified in mRNAs upstream of the polyA, but at least four variants have also been found (Wickens and Stephenson, 1984). In plants, not nearly so much analysis has been done, but it is clear that multiple sequences similar to AATAAA can be used. The plant sites in Table 2 called major or minor refer only to the study of Dean *et al.* (1986) which analyzed only three types of plant gene. The designation of polyadenylation sites as major or minor refers only to the frequency of their occurrence as functional sites in naturally occurring genes that have been analyzed. In the case of plants this is a very limited database. It is hard to predict with any certainty that a site designated major or minor is more or less likely to function partially or completely when found in a heterologous gene such as those encoding the crystal proteins of the present invention.

TABLE 2 - POLYADENYLATION SITES IN PLANT GENES

PA	AATAAA	Major consensus site
P1A	AATAAT	Major plant site
P2A	AACCAA	Minor plant site
P3A	ATATAA	"
P4A	AATCAA	"
P5A	ATACTA	"
P6A	ATAAAA	"
P7A	ATGAAA	"
P8A	AAGCAT	"
P9A	ATTAAT	"
P10A	ATACAT	"
P11A	AAAATA	"
P12A	ATTAAA	Minor animal site
P13A	AATTAA	"
P14A	AATACA	"
P15A	CATAAA	"

The present invention provides a method for preparing synthetic plant genes which genes express their protein product at levels significantly higher than the wild-type genes which were commonly employed in plant transformation heretofore. In another aspect, the present invention also provides novel synthetic plant genes which encode non-plant proteins.

As described above, the expression of native *B. thuringiensis* genes in plants is often problematic. The nature of the coding sequences of *B. thuringiensis* genes distinguishes them from plant genes as well as many other heterologous genes expressed in plants. In particular, *B. thuringiensis* genes are very rich (~62%) in adenine (A) and thymine (T) while plant genes and most other bacterial genes which have been expressed in plants are on the order of 45-55% A+T.

Due to the degeneracy of the genetic code and the limited number of codon choices for any amino acid, most of the "excess" A+T of the structural coding sequences of some *Bacillus* species are found in the third position of the codons. That is, genes of some *Bacillus* species have A or T as the third nucleotide in many codons. Thus A+T content in part can determine codon usage bias. In addition, it is clear that genes evolve for maximum function in the organism in which they evolve. This means that particular nucleotide sequences found in a gene from one organism, where they may play no role except to code for a particular stretch of amino acids, have the potential to be recognized as gene control elements in another organism (such as transcriptional promoters or terminators, polyA addition sites, intron splice sites, or specific mRNA degradation signals). It is perhaps surprising that such misread signals are not a more common feature of heterologous gene expression, but this can be explained in part by the relatively homogeneous A+T content (~50%) of many organisms. This A+T content plus the nature of the genetic code put clear constraints on the likelihood of occurrence of any particular oligonucleotide sequence. Thus, a gene from *E. coli* with a 50% A+T content is much less likely to contain any particular A+T rich segment than a gene from *B. thuringiensis*.

Typically, to obtain high-level expression of the S-endotoxin genes in plants, existing structural coding sequence ("structural gene") which codes for the S-endotoxin are modified by removal of ATTTA sequences and putative polyadenylation signals by site directed mutagenesis of the DNA comprising the structural gene. It is most preferred that substantially all the polyadenylation signals and ATTTA sequences are removed although enhanced expression levels are observed with only partial removal of either of the above identified sequences.

Alternately if a synthetic gene is prepared which codes for the expression of the subject protein, codons are selected to avoid the ATTTA sequence and putative polyadenylation signals. For purposes of the present invention putative polyadenylation signals include, but are not necessarily limited to, AATAAA, AATAAT, AACCAA, ATATAA, AATCAA, ATACTA, ATAAAA, ATGAAA, AAGCAT, ATTAAT, ATACAT, AAAATA, ATTAAA, AATTAA, AATACA and CATAAA. In replacing the ATTTA sequences and polyadenylation signals, codons are preferably utilized which avoid the codons which are rarely found in plant genomes.

The selected DNA sequence is scanned to identify regions with greater than four consecutive adenine (A) or thymine (T) nucleotides. The A+T regions are scanned for potential plant polyadenylation signals. Although the absence of five or more consecutive A or T nucleotides eliminates most plant polyadenylation signals, if there are more than one of the minor polyadenylation signals identified within ten nucleotides of each other, then the nucleotide sequence of this region is preferably altered to remove these signals while maintaining the original encoded amino acid sequence.

The second step is to consider the about 15 to about 30 or so nucleotide residues surrounding the A+T rich region identified in step one. If the A+T content of the surrounding region is less than 80%, the region should be examined for polyadenylation signals. Alteration of the region based on polyadenylation signals is dependent upon (1) the number of polyadenylation signals present and (2) presence of a major plant polyadenylation signal.

The extended region is examined for the presence of plant polyadenylation signals. The polyadenylation signals are removed by site-directed mutagenesis of the DNA sequence. The extended region is also examined for multiple copies of the ATTTA sequence which are also removed by mutagenesis.

It is also preferred that regions comprising many consecutive A+T bases or G+C bases are disrupted since these regions are predicted to have a higher likelihood to form hairpin structure due to self-complementarity. Therefore, insertion of heterogeneous base pairs would reduce the likelihood of self-complementary secondary structure formation which are known to inhibit transcription and/or translation in some organisms. In most cases, the adverse effects may be minimized by using sequences which do not contain more than five consecutive A+T or G+C.

5.8.6 Synthetic Oligonucleotides for Mutagenesis

When oligonucleotides are used in the mutagenesis, it is desirable to maintain the proper amino acid sequence and reading frame, without introducing common restriction sites such as *Bgl*II, *Hin*dIII, *Sac*I, *Kpn*I, *Eco*RI, *Nco*I, *Pst*I and *Sal*I into the modified gene. These restriction sites are found in poly-linker insertion sites of many cloning vectors. Of course, the introduction of new polyadenylation signals, ATTTA sequences or consecutive stretches of more than five A+T or G+C, should also be avoided. The preferred size for the oligonucleotides is about 40 to about 50 bases, but fragments ranging from about 18 to about 100 bases have been utilized. In most cases, a minimum of about 5 to about 8 base pairs of homology to the template DNA on both ends of the synthesized fragment are maintained to insure proper hybridization of the primer to the template. The oligonucleotides should avoid sequences longer than five base pairs A+T or G+C. Codons used in the replacement of wild-type codons should preferably avoid the TA or CG doublet wherever possible. Codons are selected from a plant preferred codon table (such as Table 3 below) so as to avoid codons which are rarely found in plant genomes, and efforts should be made to select codons to preferably adjust the G+C content to about 50%.

Regions with many consecutive A+T bases or G+C bases are predicted to have a higher likelihood to form hairpin structures due to self-complementarity. Disruption of these regions by the insertion of heterogeneous base pairs is preferred and should reduce the likelihood of the formation of self-complementary secondary structures such as hairpins which are known in some organisms to inhibit transcription (transcriptional terminators) and translation (attenuators).

Alternatively, a completely synthetic gene for a given amino acid sequence can be prepared, with regions of five or more consecutive A+T or G+C nucleotides being avoided. Codons are selected avoiding the TA and CG doublets in codons whenever possible. Codon usage can be normalized against a plant preferred codon usage table (such as Table 3) and the G+C content preferably adjusted to about 50%. The resulting sequence should be examined to ensure that there are minimal putative plant polyadenylation signals and ATTTA sequences.

Table 3 - Preferred Codon Usage in Plants

Amino Acid	Codon	Percent Usage in Plants	Amino Acid	Codon	Percent Usage in Plants
ARG	CGA	7	LEU	CUA	8
	CGC	11		CUC	20
	CGG	5		CUG	10
	CGU	25		CUU	28
	AGA	29		UUA	5
	AGG	23		UUG	30
SER	UCA	14	ALA	GCA	23
	UCC	26		GCC	32
	UCG	3		GCG	3
	UCU	21		GCU	41
	AGC	21	GLY	GGA	32
	AGU	15		GGC	20
THR	ACA	21		GGG	11
	ACC	41		GGU	37
	ACG	7	ILE	AUA	12
	ACU	31		AUC	45
PRO	CCA	45		AUU	43
	CCC	19	VAL	GUA	9
	CCG	9		GUC	20
	CCU	26		GUG	28
HIS	CAC	65		GUU	43
	CAU	35	LYS	AAA	36
GLU	GAA	48		AAG	64
	GAG	52	ASN	AAC	72
ASP	GAC	48		AAU	28
	GAU	52	GLN	CAA	64
TYR	UAC	68		CAG	36
	UAU	32	PHE	UUC	56
CYS	UGC	78		UUU	44
	UGU	22	MET	AUG	100
			TRP	UGG	100

Restriction sites found in commonly used cloning vectors are also preferably avoided. However, placement of several unique restriction sites throughout the gene is useful for analysis of gene expression or construction of gene variants.

5.8.7 "Plantized" Gene Constructs

The expression of a plant gene which exists in double-stranded DNA form involves transcription of messenger RNA (mRNA) from one strand of the DNA by RNA polymerase enzyme, and the subsequent processing of the mRNA primary transcript inside the nucleus. This processing involves a 3′ non-translated region which adds polyadenylate nucleotides to the 3′ end of the RNA. Transcription of DNA into mRNA is regulated by a region of DNA usually referred to as the "promoter." The promoter region contains a sequence of bases that signals

RNA polymerase to associate with the DNA and to initiate the transcription of mRNA using one of the DNA strands as a template to make a corresponding strand of RNA.

A number of promoters which are active in plant cells have been described in the literature. These include the nopaline synthase (NOS) and octopine synthase (OCS) promoters (which are carried on tumor-inducing plasmids of *A. tumefaciens*), the Cauliflower Mosaic Virus (CaMV) 19S and 35S promoters, the light-inducible promoter from the small subunit of ribulose bis-phosphate carboxylase (ssRUBISCO, a very abundant plant polypeptide) and the mannopine synthase (MAS) promoter (Velten *et al.*, 1984; Velten and Schell, 1985). All of these promoters have been used to create various types of DNA constructs which have been expressed in plants (see *e.g.*, Intl. Pat. Appl. Publ. Ser. No. WO 84/02913).

Promoters which are known or are found to cause transcription of RNA in plant cells can be used in the present invention. Such promoters may be obtained from plants or plant viruses and include, but are not limited to, the CaMV35S promoter and promoters isolated from plant genes such as ssRUBISCO genes. As described below, it is preferred that the particular promoter selected should be capable of causing sufficient expression to result in the production of an effective amount of protein.

The promoters used in the DNA constructs (*i.e.* chimeric plant genes) of the present invention may be modified, if desired, to affect their control characteristics. For example, the CaMV35S promoter may be ligated to the portion of the ssRUBISCO gene that represses the expression of ssRUBISCO in the absence of light, to create a promoter which is active in leaves but not in roots. The resulting chimeric promoter may be used as described herein. For purposes of this description, the phrase "CaMV35S" promoter thus includes variations of CaMV35S promoter, *e.g.*, promoters derived by means of ligation with operator regions, random or controlled mutagenesis, *etc.* Furthermore, the promoters may be altered to contain multiple "enhancer sequences" to assist in elevating gene expression.

The RNA produced by a DNA construct of the present invention also contains a 5′ non-translated leader sequence. This sequence can be derived from the promoter selected to express the gene, and can be specifically modified so as to increase translation of the mRNA. The 5′ non-translated regions can also be obtained from viral RNA's, from suitable eukaryotic genes, or from a synthetic gene sequence. The present invention is not limited to constructs, as presented in the following examples. Rather, the non-translated leader sequence can be part of the 5′ end

of the non-translated region of the coding sequence for the virus coat protein, or part of the promoter sequence, or can be derived from an unrelated promoter or coding sequence. In any case, it is preferred that the sequence flanking the initiation site conform to the translational consensus sequence rules for enhanced translation initiation reported by Kozak (1984).

The *cry* DNA constructs of the present invention may also contain one or more modified or fully-synthetic structural coding sequences which have been changed to enhance the performance of the *cry* gene in plants. The structural genes of the present invention may optionally encode a fusion protein comprising an amino-terminal chloroplast transit peptide or secretory signal sequence.

The DNA construct also contains a 3′ non-translated region. The 3′ non-translated region contains a polyadenylation signal which functions in plants to cause the addition of polyadenylate nucleotides to the 3′ end of the viral RNA. Examples of suitable 3′ regions are (1) the 3′ transcribed, non-translated regions containing the polyadenylation signal of *Agrobacterium* tumor-inducing (Ti) plasmid genes, such as the nopaline synthase (NOS) gene, and (2) plant genes like the soybean storage protein (7S) genes and the small subunit of the RuBP carboxylase (E9) gene.

5.9 Methods for Producing Insect-Resistant Transgenic Plants

By transforming a suitable host cell, such as a plant cell, with a recombinant *cryET31, cryET40, cryET43, cryET44, cryET45, cryET46, cryET47, cryET49, cryET51, cryET52, cryET53, cryET54, cryET56, cryET57, cryET59, cryET60, cryET61, cryET62, cryET63, cryET64, cryET66, cryET67, cryET68, cryET72, cryET73,* and *cryET83* gene-containing segment, the expression of the encoded crystal protein (*i.e.*, a bacterial crystal protein or polypeptide having insecticidal activity against coleopterans) can result in the formation of insect-resistant plants.

By way of example, one may utilize an expression vector containing a coding region for a *B. thuringiensis* crystal protein and an appropriate selectable marker to transform a suspension of embryonic plant cells, such as wheat or corn cells using a method such as particle bombardment (Maddock *et al.*, 1991; Vasil *et al.*, 1992) to deliver the DNA coated on microprojectiles into the recipient cells. Transgenic plants are then regenerated from transformed embryonic calli that express the insecticidal proteins.

The formation of transgenic plants may also be accomplished using other methods of cell transformation which are known in the art such as *Agrobacterium*-mediated DNA transfer (Fraley *et al.*, 1983). Alternatively, DNA can be introduced into plants by direct DNA transfer into pollen (Zhou *et al.*, 1983; Hess, 1987; Luo *et al.*, 1988), by injection of the DNA into reproductive organs of a plant (Pena *et al.*, 1987), or by direct injection of DNA into the cells of immature embryos followed by the rehydration of desiccated embryos (Neuhaus *et al.*, 1987; Benbrook *et al.*, 1986).

The regeneration, development, and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach and Weissbach, 1988). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a polypeptide of interest introduced by *Agrobacterium* from leaf explants can be achieved by methods well known in the art such as described (Horsch *et al.*, 1985). In this procedure, transformants are cultured in the presence of a selection agent and in a medium that induces the regeneration of shoots in the plant strain being transformed as described (Fraley *et al.*, 1983).

This procedure typically produces shoots within two to four months and those shoots are then transferred to an appropriate root-inducing medium containing the selective agent and an antibiotic to prevent bacterial growth. Shoots that rooted in the presence of the selective agent to form plantlets are then transplanted to soil or other media to allow the production of roots. These procedures vary depending upon the particular plant strain employed, such variations being well known in the art.

Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants, as discussed before. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important, preferably inbred lines. Conversely, pollen from plants of those important lines is used to pollinate regenerated plants. A transgenic plant of the

present invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

A transgenic plant of this invention thus has an increased amount of a coding region (*e.g.*, a *cryET31, cryET40, cryET43, cryET44, cryET45, cryET46, cryET47, cryET49, cryET51, cryET52, cryET53, cryET54, cryET56, cryET57, cryET59, cryET60, cryET61, cryET62, cryET63, cryET64, cryET66, cryET67, cryET68, cryET72, cryET73,* and *cryET83* gene) that encodes one or more CryET31, CryET40, CryET43, CryET44, CryET45, CryET46, CryET47, CryET49, CryET51, CryET52, CryET53, CryET54, CryET56, CryET57, CryET59, CryET60, CryET61, CryET62, CryET63, CryET64, CryET66, CryET67, CryET68, CryET72, CryET73, and CryET83 polypeptides. A preferred transgenic plant is an independent segregant and can transmit that gene and its activity to its progeny. A more preferred transgenic plant is homozygous for that gene, and transmits that gene to all of its offspring on sexual mating. Seed from a transgenic plant may be grown in the field or greenhouse, and resulting sexually mature transgenic plants are self-pollinated to generate true breeding plants. The progeny from these plants become true breeding lines that are evaluated for, by way of example, increased insecticidal capacity against coleopteran insects, preferably in the field, under a range of environmental conditions. The inventors contemplate that the present invention will find particular utility in the creation of transgenic plants of commercial interest including various turf grasses, wheat, corn, rice, barley, oats, a variety of ornamental plants and vegetables, as well as a number of nut- and fruit-bearing trees and plants.

5.10 Definitions

The following words and phrases have the meanings set forth below.

Expression: The combination of intracellular processes, including transcription and translation undergone by a coding DNA molecule such as a structural gene to produce a polypeptide.

Identity or **percent identity**: refers to the degree of similarity between two nucleic acid or protein sequences. An alignment of the two sequences is performed by a suitable computer program. A widely used and accepted computer program for performing sequence alignments is CLUSTALW v1.6 (Thompson, et al. *Nucl. Acids Res.*, 22: 4673-4680, 1994). The number of matching bases or amino acids is divided by the total number of bases or amino acids, and multiplied by 100 to obtain a percent identity. For example, if two 580 base pair sequences had

145 matched bases, they would be 25 percent identical. If the two compared sequences are of different lengths, the number of matches is divided by the shorter of the two lengths. For example, if there were 100 matched amino acids between 200 and a 400 amino acid proteins, they are 50 percent identical with respect to the shorter sequence. If the shorter sequence is less than 150 bases or 50 amino acids in length, the number of matches are divided by 150 (for nucleic acid bases) or 50 (for amino acids), and multiplied by 100 to obtain a percent identity.

Promoter: A recognition site on a DNA sequence or group of DNA sequences that provide an expression control element for a structural gene and to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that gene.

Regeneration:The process of growing a plant from a plant cell (*e.g.*, plant protoplast or explant).

Structural gene: A polynucleotide sequence that encodes a polypeptide, that is expressed to produce a polypeptide, or which is cryptic or incapable of expression in its natural host cell but which can be isolated and purified and operably linked to at least a promoter functional in one or more host cell types to express the encoded polypeptide.

Transformation: A process of introducing an exogenous DNA sequence (*e.g.*, a vector, a recombinant DNA molecule) into a cell or protoplast in which that exogenous DNA is incorporated into a chromosome or is capable of autonomous replication.

Transformed cell: A cell whose DNA has been altered by the introduction of an exogenous DNA molecule into that cell.

Transgenic cell: Any cell derived or regenerated from a transformed cell or derived from a transgenic cell. Exemplary transgenic cells include plant calli derived from a transformed plant cell and particular cells such as leaf, root, stem, *e.g.*, somatic cells, or reproductive (germ) cells obtained from a transgenic plant.

Transgenic plant: A plant or progeny thereof derived from a transformed plant cell or protoplast, wherein the plant DNA contains an introduced exogenous DNA molecule not originally present in a native, non-transgenic plant of the same strain. The terms "transgenic plant" and "transformed plant" have sometimes been used in the art as synonymous terms to define a plant whose DNA contains an exogenous DNA molecule. However, it is thought more scientifically correct to refer to a regenerated plant or callus obtained from a transformed plant cell or protoplast as being a transgenic plant, and that usage will be followed herein.

Vector: A DNA molecule capable of replication in a host cell and/or to which another DNA segment can be operatively linked so as to bring about replication of the attached segment. A plasmid is an exemplary vector.

5.11 Isolating Homologous Gene and Gene Fragments

The genes and δ-endotoxins according to the subject invention include not only the full length sequences disclosed herein but also fragments of these sequences, or fusion proteins, which retain the characteristic insecticidal activity of the sequences specifically exemplified herein.

It should be apparent to a person skill in this art that insecticidal δ-endotoxins can be identified and obtained through several means. The specific genes, or portions thereof, may be obtained from a culture depository, or constructed synthetically, for example, by use of a gene machine. Variations of these genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as *Bal*31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which code for active fragments may be obtained using a variety of other restriction enzymes. Proteases may be used to directly obtain active fragments of these δ-endotoxins.

Equivalent δ-endotoxins and/or genes encoding these equivalent δ-endotoxins can also be isolated from *Bacillus* strains and/or DNA libraries using the teachings provided herein. For example, antibodies to the δ-endotoxins disclosed and claimed herein can be used to identify and isolate other δ-endotoxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the δ-endotoxins which are most constant and most distinct from other *B. thuringiensis* δ-endotoxins. These antibodies can then be used to specifically identify equivalent δ-endotoxins with the characteristic insecticidal activity by immunoprecipitation, enzyme linked immunoassay (ELISA), or Western blotting.

A further method for identifying the δ-endotoxins and genes of the subject invention is through the use of oligonucleotide probes. These probes are nucleotide sequences having a detectable label. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong bond between the two molecules, it can be reasonably assumed

that the probe and sample are essentially identical. The probe's detectable label provides a means for determining in a known manner whether hybridization has occurred. Such a probe analysis provides a rapid method for identifying formicidal δ-endotoxin genes of the subject invention.

The nucleotide segments which are used as probes according to the invention can be synthesized by use of DNA synthesizers using standard procedures. In the use of the nucleotide segments as probes, the particular probe is labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels. Typical radioactive labels include ^{32}P, ^{125}I, ^{35}S, or the like. A probe labeled with a radioactive isotope can be constructed from a nucleotide sequence complementary to the DNA sample by a conventional nick translation reaction, using a DNase and DNA polymerase. The probe and sample can then be combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs. Thereafter, the membrane is washed free of extraneous materials, leaving the sample and bound probe molecules typically detected and quantified by autoradiography and/or liquid scintillation counting.

Non-radioactive labels include, for example, ligands such as biotin or thyroxine, as well as enzymes such as hydrolases or peroxidases, or the various chemiluminescers such as luciferin, or fluorescent compounds like fluorescein and its derivatives. The probe may also be labeled at both ends with different types of labels for ease of separation, as, for example, by using an isotopic label at the end mentioned above and a biotin label at the other end.

Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid, and, as noted above, a certain degree of mismatch can be tolerated. Therefore, the probes of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence in many ways, by methods currently known to an ordinarily skilled artisan, and perhaps by other methods which may become known in the future.

The potential variations in the probes listed is due, in part, to the redundancy of the genetic code. Because of the redundancy of the genetic code, *i.e.*, more than one coding nucleotide triplet (codon) can be used for most of the amino acids used to make proteins.

Therefore different nucleotide sequences can code for a particular amino acid. Thus, the amino acid sequences of the *B. thuringiensis* δ-endotoxins and peptides can be prepared by equivalent nucleotide sequences encoding the same amino acid sequence of the protein or peptide. Accordingly, the subject invention includes such equivalent nucleotide sequences. Also, inverse or complement sequences are an aspect of the subject invention and can be readily used by a person skilled in this art. In addition it has been shown that proteins of identified structure and function may be constructed by changing the amino acid sequence if such changes do not alter the protein secondary structure (Kaiser and Kezdy, 1984). Thus, the subject invention includes mutants of the amino acid sequence depicted herein which do not alter the protein secondary structure, or if the structure is altered, the biological activity is substantially retained. Further, the invention also includes mutants of organisms hosting all or part of a δ-endotoxin encoding a gene of the invention. Such mutants can be made by techniques well known to persons skilled in the art. For example, UV irradiation can be used to prepare mutants of host organisms. Likewise, such mutants may include asporogenous host cells which also can be prepared by procedures well known in the art.

6.0 Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

6.1 Example 1 -- Identification of *B. thuringiensis* Strains Containing Novel δ -Endotoxins

Wild-type *B. thuringiensis* strains containing novel insecticidal protein genes were identified by Southern blot hybridization studies employing specific DNA probes. Twenty-four unique *cry* genes were discovered that are related to *B. thuringiensis* genes in the *cry1, cry2,* or *cry9* classes of toxin genes.

Various methods were employed to clone the novel genes and express them in a crystal protein-negative (Cry-) strain of *B. thuringiensis.* These methods include PCR™ amplification of the region of *cry1*-related genes that encodes the active portion of the toxin gene. The PCR™ product is then joined to a fragment from the *cry1Ac* gene encoding the C-terminal region of the protoxin. This gene fusion was then expressed in a *B. thuringiensis* recombinant strain to produce a hybrid protoxin. In this instance, it is recognized that the sequence of the amplified DNA can be used to design hybridization probes to isolate the entire coding sequence of the novel *cry* gene from the wild-type *B. thuringiensis* strain.

Wild-type *B. thuringiensis* strains were screened in a bioassay to identify strains that are toxic to larvae of lepidopteran insects (procedure described in Example 10). Active strains were then examined genetically to determine if they contain novel toxin genes. The method used to make this determination is described below and includes isolation of genomic DNA from the *B. thuringiensis* strain, restriction enzyme digestion, Southern blot hybridization, and analysis of the hybridizing restriction fragments to determine which genes are present in a strain.

Total genomic DNA was extracted by the following procedure. Vegetative cells were resuspended in a lysis buffer containing 50 mM glucose, 25 mM Tris-HCl (pH 8.0), 10 mM EDTA, and 4 mg/ml lysozyme. The suspension was incubated at 37°C for 1 h. Following incubation, the suspension was extracted once with an equal volume of phenol, then once with an equal volume of phenol:chloroform:isoamyl alcohol (50:48:2), and once with an equal volume of chloroform:isoamyl alcohol (24:1). The DNA was precipitated from the aqueous phase by the addition of one-tenth volume 3 M sodium acetate and two volumes of 100% ethanol. The precipitated DNA was collected by centrifugation, washed with 70% ethanol and resuspended in distilled water.

The DNA samples were digested with the restriction enzymes *Cla*I and *Pst*I. The combination of these two enzymes give a digestion pattern of fragments that, when hybridized with the probe wd207 (described below), allows the identification of many of the known *cry1*-related toxin genes. Hybridizing fragments that did not correspond to the fragment sizes expected for the known genes were classified as unknown and were candidates for cloning and characterization.

The digested DNA was size fractionated by electrophoresis through a 1.0% agarose gel in 1X TBE (0.089 M Tris-borate, 0.089 M boric acid, 0.002 M EDTA) overnight at 2 V/cm of gel

length. The fractionated DNA fragments were then transferred to a Millipore Immobilon-NC® nitrocellulose filter (Millipore Corp., Bedford, MA) according to the method of Southern (1975). The DNA fragments were fixed to the nitocellulose by baking the filter at 80°C in a vacuum oven.

To identify the DNA fragment(s) containing the sequences related to *cryI* genes, the oligonucleotide wd207 was radioactively labeled at the 5' end and used as a hybridization probe. To radioactively label the probe, 1-5 pmoles of wd207 were added to a reaction (20 ul total volume) containing 3 ul [γ-^{32}P]ATP (3,000 Ci/mmole at 10 mCi/ml), 70 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, 5 mM DTT, and 10 units T4 polynucleotide kinase (Promega Corp., Madison, WI). The reaction was incubated for 20 min at 37°C to allow the transfer of the radioactive phosphate to the 5'-end of the oligonucleotide, thus making it useful as a hybridization probe.

The oligonucleotide probe used in this analysis, designated wd207, has the following sequence:

5'-TGGATACTTGATCAATATGATAATCCGTCACATCTGTTTTTA-3' (SEQ ID NO:51)

This oligonucleotide was designed to specifically hybridize to a conserved region of *cryI* genes downstream from the proteolyic activation site in the protoxin. Table 4 lists some of the *B. thuringiensis* toxin genes and their identities with wd207. The orientation of the wd207 sequence is inverted and reversed relative to the coding sequences of the *cry* genes.

TABLE 4

cry Gene	% Identity to wd207	Nucleotide Position in CDS
cryIAa	100%	1903-1944
cryIBa	95.2%	1991-2032
cryICa	97.6%	1930-1971
cryIDa	97.6%	1858-1899
cryIEa	97.6%	1885-1926

The labeled probe was then incubated with the nitrocellulose filter overnight at 45°C in 3X SSC (1X SSC = 0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS, 10X Denhardt's reagent (0.2% BSA, 0.2% polyvinylpyrrolidone, 0.2% Ficoll), and 0.2 mg/ml heparin. Following this incubation period, the filter was washed in several changes of 3X SSC, 0.1% SDS at 45°C. The filter was blotted dry and exposed to Kodak X-OMAT AR X-ray film (Eastman Kodak Co., Rochester, NY) overnight at -70°C with an intensifying screen to obtain an autoradiogram.

The autoradiograms were analyzed to determine which wild-type *B. thuringiensis* strains contained *cry1* genes that could be novel. Since the probe was only 42 nucleotides, it is unlikely that recognition sites for the restriction endonucleases *Cla*I and *Pst*I would occur within the hybridizing region of the *cry1*-related genes. Therefore, it was assumed that each hybridizing restriction fragment represented one *cry1*-related gene. The sizes, in kilobases (kb), of the hybridizing restriction fragments were determined based on the migration of the fragment in the agarose gel relative to DNA fragments of known size. The size of a fragment could be used to determine if that fragment represented a known *cry1* gene. For example, from the DNA sequence of the *cry1Ac* gene it was known that wd207 would hybridize to a 0.43 kb fragment after digestion of *cry1Ac* DNA with *Cla*I and *Pst*I. If the Southern blot analysis of a strain showed a 0.43 kb hybrizing fragment, that strain was assigned a probable genotype of *cry1Ac*. Fragments that could not be easily assigned a probable genotype were selected as candidates for further analysis. Because many *cry1*-containing strains have more than one *cry1*-related gene, all fragments were given a putative designation.

TABLE 5 - SUMMARY OF GENES AND PROTEINS

Polypeptide Designation	Polypeptide Seq. ID No.:	Polynucleotide Seq ID No.:	WT-Strain	Recomb. Strain	Gene Family	Cloning Method[1]	DNA Probe[2]	Cloning Vector	Plasmid
Cry ET31	2	1	EG6701	EG11562	*cry2*	*Mbo*I	*cry2a*	pHT315	pEG1331
Cry ET40	4	3	EG5476	EG11901	*cry1*	PCR™	-	pEG1064	pEG1901
Cry ET43	6	5	EG2878	EG7692	*cry1*	PCR™	-	pEG1064	pEG1806
Cry ET44	8	7	EG3114	EG11629	*cry1*	PCR™	-	pEG1064	pEG1807
Cry ET45	10	9	EG3114	EG7694	*cry1*	PCR™	-	pEG1064	pEG1808
Cry ET46	12	11	EG6451	EG7695	*cry1*	PCR™	-	pEG1064	pEG1809
Cry ET47	14	13	EG6451	EG7696	*cry1*	PCR™	-	pEG1064	pEG1810
Cry ET49	16	15	EG6451	EG11630	*cry1*	PCR™	-	pEG1064	pEG1812
Cry ET51	18	17	EG5391	EG11921	*cry1*	*Mbo*I	wd207	pHT315	pEG1912
Cry ET52	20	19	EG10475	EG11584	*cry1*	*Bam*HI	wd207	pEG290	pEG1340
Cry ET53	22	21	EG3874	EG11906	*cry1*	*Mbo*I	*cry1Aa*	pHT315	pEG1904
Cry ET54			EG3874	EG11907	*cry1*	*Mbo*I	*cry1Aa*	pHT315	pEG1905
Cry ET56	24	23	EG3874	EG11909	*cry1*	*Mbo*I	*cry1Aa*	pHT315	pEG1907
Cry ET57	26	25	EG3874	EG11910	*cry1*	*Mbo*I	*cry1Aa*	pHT315	pEG1908
Cry ET59	28	27	EG9290	EG12102	*cry9*	*Mbo*I	pr56, *cryET59*	pHT315	pEG945
Cry ET60	30	29	EG9290	EG12103	*cry9*	*Mbo*I	pr56, *cryET59*	pHT315	pEG946
Cry ET61	32	31	EG4612	EG11634	*cry1*	*Mbo*I	wd207	pHT315	pEG1813
Cry ET62	34	33	EG6831	EG11635	*cry1*	*Mbo*I	wd207	pHT315	pEG1814
Cry ET63	36	35	EG4623	EG11636	*cry1*	*Mbo*I	wd207	pHT315	pEG1815
Cry ET64	38	37	EG4612	EG11638	*cry1*	*Mbo*I	wd207	pHT315	pEG1816
Cry ET66	40	39	EG5020	EG11640	*cry1*	*Mbo*I	wd207	pHT315	pEG1817
Cry ET67	42	41	EG4869	EG11642	*cry1*	*Mbo*I	wd207	pHT315	pEG1818
Cry ET68	44	43	EG5020	EG11644	*cry1*	*Mbo*I	wd207	pHT315	pEG1819
Cry ET72	46	45	EG4420	EG11440	*cry2*	*Hind*III	*cry2Aa*	pEG597	pEG1260
Cry ET73	48	47	EG3874	EG11465	*cry2*	*Hind*III	*cry2Aa*	pEG597	pEG1279
Cry ET83	50	49	EG6346	EG11785	*cry9*	*Mbo*I	*cryET59, cryET83*	pHT315	pEG397

[1] Methods include the construction of genomic libraries containing partial *Mbo*I fragments (Example 4), the construction of genomic libraries containing size-selected *Bam*HI or *Hind*III restriction fragments (Example 5), the amplification of novel *cry* sequences by PCR™ and the construction of novel *cry* gene fusions (Example 6).

[2] Hybridization probes included the 700 base pair *Eco*RI fragment obtained from digestion of the *cry1Aa* gene, gene fragments from the *cry2Aa, cryET59, and cryET83* genes, and synthetic oligonucleotides (wd207, pr56).

6.2 EXAMPLE 2 -- IDENTIFICATION *OF B. THURINGIENSIS* STRAINS CONTAINING NOVEL *CRY2*-RELATED GENES

Proteins encoded by the *cry2* class of *B. thuringiensis* class of toxin genes have activity on the larvae of lepidopteran and diopteran insects. Southern blot hybridization analysis of DNA extracted from lepidopteran-active strains was utilized to identify novel *cry2*-related genes. Total genomic DNA was isolated as described in Section 6.1. The DNA was digested with the restriction endonuclease *Sau3A* and run on a 1.2% agarose gel as described. The digested DNA was transferred to nitrocellulose filters to be probed with a DNA fragment containing the *cry2Aa* gene. Hybridizations were performed at 55°C and the filters washed and exposed to X-ray film to obtain an autoradiogram.

Sau3A digestion followed by hybridization with the *cry2Aa* gene gave characteristic patterns of hybridizing fragments allowing the identification of the *cry2Aa, cry2Ab, and cry2Ac* genes. Hybridizing fragments that differed from these patterns indicated the presence of a novel *cry2*-related gene in that strain.

Once a strain was identified as containing one or more novel *cry2*-related genes, an additional Southern blot hybridization was performed. The procedures were the same as those already described above, except another restriction enzyme, usually *Hin*dIII, was used. Since an enzyme like *Hin*dIII (a "six base cutter") cuts DNA less frequently than does *Sau*3A or *Mbo*I, it was more likely to generate a restriction fragment containing the entire *cry2*-related gene which could then be readily cloned.

6.3 Example 3 -- Identification of *B. thuringiensis* Strains Containing Novel *cry9*-Type Genes

A *cry9*-specific oligonucleotide, designated pr56, was designed to facilitate the identification of strains harboring *cry9*-type genes. This oligonucleotide corresponds to nucleotides 4349-4416 of the gene (GenBank Accession No. Z37527). The sequence of pr56 was as follows:

5′-AGTAACGGTGTTACTATTAGCGAGGGCGGTCCATTCTTTAA AGGTCGTGCACTTCAGTTAGC-3′ (SEQ ID NO:52).

B. thuringiensis isolates were spotted or "patched" on SGNB plates, with no more than 50 isolates per plate, and grown overnight at 25°C. The *B. thuringiensis* colonies were transferred to nitrocellulose filters and the filters placed, colony side up, on fresh SGNB plates for overnight

growth at 30°C. Subsequently, the filters were placed, colony side up, on Whatman paper soaked in denaturing solution (1.5 M NaCl, 0.5 N NaOH) for 20 min. After denaturation, the filters were placed on Whatman paper soaked in neutralizing solution (3 M NaCl, 1.5 M Tris-HCl, pH 7.0) for 20 min. Finally, the filters were washed in 3X SSC (1X SSC = 0.15 M NaCl and 0.015 M sodium citrate) to remove cellular debris and baked in a vacuum oven at 80°C for 90 min.

The *cry9*-specific oligonucleotide pr56 (~10 pmoles) was end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase. The labeling reaction was carried out at 37°C for 20 min and terminated by incubating the reaction at 100 C for 3 min. After ethanol precipitation, the labeled oligonucleotide was resuspended in 100 µl distilled H_2O.

The filters were incubated with the *cry9*-specific probe in 6X SSC, 10X Denhardt's solution, 0.5% glycine, 0.2% SDS at 47°C overnight. The filters were washed twice in 3X SSC, 0.1% SDS for 15 min at 47°C and twice in 1X SSC, 0.1% SDS for 15 min at 47°C. The dried filters were exposed to X-OMAT XAR-5 film (Eastman Kodak Co.) at -70°C using an intensifying screen. The developed autoradiogram revealed 24 isolates of *B. thuringiensis* containing DNA that hybridized to the *cry9* probe.

To identify *cry9C*-type genes among these strains, two opposing oligonucleotide primers specific for the *cry9C* gene (GenBank Accession No. Z37527) were designed for polymerase chain reaction (PCR™) analyses. The sequence of pr58 is:

5'-CGACTTCTCCTGCTAATGGAGG-3' (SEQ ID NO:53).

The sequence of pr59 is:

5'-CTCGCTAATAGTAACACCGTTACTTGCC-3' (SEQ ID NO:54).

Plasmid DNAs were isolated from the isolates of *B. thuringiensis* believed to contain *cry9*-type genes. *B. thuringiensis* isolates were grown overnight at 30°C on Luria agar plates and 2 loopfuls of cells from each isolate were suspended in 50 mM glucose, 10 mM Tris-HCl, 1 mM EDTA (1X GTE) containing 4 mg/ml lysozyme. After a 10 min incubation at room temperature, plasmid DNAs were extracted using a standard alkaline lysis procedure (Maniatis *et al.*, 1982). The plasmid DNAs were resuspended in 20 µl of 1X TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). Two microliters of the plasmid DNA preparations were used in the PCR™ reactions. Amplifications were performed in 100 µl volumes with a Perkin-Elmer DNA Thermocycler (Perkin-Elmer Cetus, Foster City, CA) using materials and methods provided in the Perkin-

Elmer GeneAmp™ kit. Conditions for the PCR™ were as follows: 95°C for 30 sec, 46°C for 30 sec, 70°C for 1 min; 30 cycles. A PCR™ using these primers and the *cry9C* gene as a template should yield a DNA fragment of ~970 bp. Of twenty-four strains found to hybridize to the *cry9* probe (SEQ ID NO:XX), only one strain, EG9290, yielded the predicted amplified DNA fragment.

6.4 EXAMPLE 4 -- CLONING OF *B. THURINGIENSIS* TOXIN GENES BY CONSTRUCTING *MBO*I PARTIAL DIGEST LIBRARIES

The restriction endonuclease *Mbo*I was utilized in the construction of genomic DNA libraries because it has a recognition sequence of four base pairs which occurs frequently in long stretches of DNA. Total genomic DNA was isolated from *B. thuringiensis* strains as described in Section 6.1. The DNA was digested under conditions allowing limited cleavage of a DNA strand. The method of establishing these conditions has been described (Maniatis *et al.*, 1982). Digestion of DNA in this manner created a set of essentially randomly cleaved, overlapping fragments which were used to create a library representative of the entire genome.

The digested DNA fragments were separated, according to size, by agarose gel electrophoresis through a 0.6% agarose, 1X TBE gel, overnight at 2 volts/cm of gel length. The gel was stained with ethidium bromide so that the digested DNA could be visualized when exposed to long-wave UV light. A razor blade was used to excise a gel slice containing DNA fragments of approximately 9- kb to 12-kb in size. The DNA fragments were removed from the agarose by placing the slice in a dialysis bag with enough TE (10 mM Tris-HCl, 1 mM EDTA) to cover the slice. The bag was then closed and placed in a horizontal electrophoresis apparatus filled with 1X TBE buffer. The DNA was electroeluted from the slice into the TE at 100 volts for 2 h. The TE was removed from the bag, extracted with phenol:chloroform (1:1), followed by extraction with chloroform. The DNA fragments are then collected by the standard technique of ethanol precipitation (see Maniatis *et al.*, 1982).

To create a library in *E. coli* of the partially-digested DNA, the fragments were ligated into the shuttle vector, pHT315 (Arantes and Lereclus, 1991). This plasmid contains replication origins for *E. coli and B. thuringiensis,* genes for resistance to the antibiotics erythromycin and ampicillin, and a multiple cloning site. The *Mbo*I fragments were mixed with *Bam*HI-digested pHT315 that had been treated with calf intestinal, or bacterial, alkaline phosphatase (GibcoBRL, Gaithersburg, MD) to remove the 5'-phosphates from the digested plasmid, preventing re-

ligation of the vector to itself. After purification, T4 ligase and a ligation buffer (Promega Corp., Madison, WI) were added to the reaction containing the digested vector and the *Mbo*I fragments. These were incubated overnight at 15°C, or at room temperature for 1 h, to allow the insertion and ligation of the *Mbo*I fragments into the pHT315 vector DNA.

The ligation mixture was then introduced into transformation-competent *E. coli* SURE® cells (Stratagene Cloning Systems, La Jolla, CA), following procedures described by the manufacturer. The transformed *E. coli* cells were then plated on LB agar plates containing 50-75 µg/ml ampicillin and incubated ovenight at 37°C. The growth of several hundred ampicillin-resistant colonies on each plate indicated the presence of recombinant plasmid in the cells of each of those colonies.

To isolate the colonies harboring sequences encoding toxin genes, the colonies were first transferred to nitrocellulose filters. This was accomplished by simply placing a circular nitrocellulose filter (Millipore HATF 08525, Millipore Corp., Bedford, MA) directly on top of the LB-ampicillin agar plates containing the transformed colonies. When the filter was slowly peeled off of the plate the colonies stick to the filter giving an exact replica of the pattern of colonies from the original plate. Enough cells from each colony were left on the plate that 5 to 6 h of growth at 37°C restored the colonies. The plates were then stored at 4°C until needed. The nitrocellulose filters with the transferred colonies are then placed, colony-side up, on fresh LB-ampicillin agar plates and allowed to grow at 37°C until they reached an approximate 1 mm diameter.

To release the DNA from the recombinant *E. coli* cells the nitrocellulose filters were placed, colony-side up, on 2-sheets of Whatman 3MM chromatogrphy paper (Whatman International Ltd., Maidstone, England) soaked with 0.5 N NaOH, 1.5 M NaCl for 15 min. This treatment lysed the cells and denatured the released DNA allowing it to stick to the nitrocellulose filter. The filters were then neutralized by placing the filters, colony-side up, on 2 sheets of Whatman paper soaked with 1 M NH_4-acetate, 0.02 M NaOH for 10 min. The filters were rinsed in 3X SSC, air dried, and baked for 1 h at 80°C in a vacuum oven. The filters were then ready for use in hybridization studies using probes to identify different classes of *B. thuringiensis* genes, as described in the above examples.

In order to identify colonies containing cloned *cryI*-related genes, the *cryI*-specific oligonucleotide wd207 was labeled at the 5'-end with [γ-^{32}P]ATP and T4 polynucleotide kinase.

The labeled probe was added to the filters in 3X SSC, 0.1% SDS, 10X Denhardt's reagent (0.2% BSA, 0.2% polyvinylpyrrolidone, 0.2% Ficoll), 0.2 mg/ml heparin and incubated overnight at 47°C. These conditions allowed hybridization of the labeled oligonucleotide to related sequences present on the nitrocellulose blots of the transformed *E. coli* colonies. Following incubation the filters were washed in several changes of 3X SSC, 0.1% SDS at 45°C. The filters were blotted dry and exposed to Kodak X-OMAT AR X-ray film (Eastman Kodak Co., Rochester, NY) overnight at -70°C with an intensifying screen.

Colonies that contain cloned *cryI*-related sequences were identified by aligning signals on the autoradiogram with the colonies on the original transformation plates. The isolated colonies were then grown in LB-ampicillin liquid medium from which the cells could be harvested and recombinant plasmid prepared by the standard alkaline-lysis miniprep procedure (Maniatis *et al.*, 1982). The plasmid DNA was then used as a template for DNA sequencing reactions necessary to confirm that the cloned gene was novel. If the cloned gene was novel, the plasmid was then introduced into a crystal protein-negative strain of *B. thuringiensis* (*Cry*$^-$) so that the encoded protein could be expressed and characterized. These procedures are described in detail in the following sections.

6.5 Example 5 -- Cloning of Specific Endonuclease Restriction Fragments

The identification of a specific restriction fragment containing a novel *B. thuringiensis* gene has been described for *cry2*-related genes in Section 2. The procedure for cloning a restriction fragment of known size was essentially the same as described for cloning an *Mbo*I fragment. The DNA was digested with a restriction enzyme (*e.g.*, *Hin*dIII), and run through an agarose gel to separate the fragments by size. Fragments of the proper size, identified by Southern blot analysis (Example 2), were excised with a razor blade and electroeluted from the gel slice into TE buffer from which they could be precipitated. The isolated restriction fragments were then ligated into an *E. coli/B. thuringiensis* shuttle vector and transformed into *E. coli* to construct a size-selected library. The library could then be hybridized with a specific gene probe, as described in Example 4, to isolate the colony containing the cloned novel gene.

6.6 Example 6 -- Cloning of PCR™-Amplified Fragments

A rapid method for cloning and expressing novel *cryI* gene fragments from *B. thuringiensis* was developed using the polymerase chain reaction. Flanking primers were

designed to anneal to conserved regions 5′ to and within *cry1* genes. With the exception of certain *cry3* genes, most *B. thuringiensis cry* genes are transcriptionally regulated, at least in part, by RNA polymerases containing the mother cell-specific σ^E or *sigE*, sigma factor. These σ^E-regulated *cry* genes possess 5′ promoter sequences that are recognized by σ^E. Alignment of these promoter sequences reveals considerable sequence variation, although a consensus sequence can be identified (Baum and Malvar, 1995). A primer, designated "*sigE*", containing a sequence identical to the *cry1Ac* σ^E promoter sequence, was designed that would anneal to related σ^E promoter sequences 5′ to uncharacterized *cry* genes. The *sigE* primer also includes a *Bbu*I site (isoschizimer: *Sph*I) to facilitate cloning of amplified fragments. The sequence of the *sigE* primer is shown below:

5′-ATTTAGTAGCATGCGTTGCACTTTGTGCATTTTTTCATAAGATGA

GTCATATGTTTTAAAT-3′ (SEQ ID NO:55).

The opposing primer, designated KpnR, anneals to a 3′-proximal region of the *cry1* gene that is generally conserved. This primer incorporates an *Asp*718 site (isoschizimer: *Kpn*I) conserved among the *cry1A* genes to facilitate cloning of the amplified fragment and to permit the construction of fusion proteins containing a carboxyl-terminal portion of the Cry1Ac protein. The sequence of the *Kpn*R primer is shown below:

5′-GGATAGCACTCATCAAAGGTACC-3′ (SEQ ID NO:56)

PCR™s were carried out using a Perkin Elmer DNA thermocycler and the following parameters: 94°C, 2 min.; 40 cycles consisting of 94°C, 30 sec; 40°C, 2 min; 72°C, 3 min; and a 10 second extension added to the 72°C incubation after 20 cycles. The standard PCR™ buffer (100 µl volume) was modified to include 1X Taq Extender buffer, 25 µM each of the *sigE* and KpnR primers, and 0.5 - 1.0 µl of Taq Extender (Stratagene Inc.) in addition to 0.5 - 1.0 µl of Taq polymerase. Typically, 1-2 µl of the DNA preparations from novel *B. thuringiensis* isolates were included in the PCR™s. PCR™s with *cry* genes incorporating these primers resulted in the amplification of a ~2.3-kb DNA fragment flanked by restriction sites for *Bbu*I *and Asp*718.

For the cloning and expression of these gene fragments, the *cry1Ac* shuttle vector pEG1064 was used. This plasmid is derived from the *cry1Ac* shuttle vector pEG857 (Baum *et al.*, 1990), with the following modifications. A frameshift mutation was generated at a unique *Nco*I site within the *cry1Ac* coding region by cleaving pEG857 with the restriction endonuclease *Nco*I, blunt-ending the *Nco*I-generated ends with Klenow polymerase and ligating the blunt ends

with T4 ligase. In similar fashion, an *Asp*718 site located in the multiple cloning site 3' to the *cry1Ac* gene was removed, leaving only the single *Asp*718 site contained within the *cry1Ac* coding sequence. The resulting plasmid, pEG1064, cannot direct the production of crystal protein when introduced into an acrystalliferous (Cry$^-$) strain of *B. thuringiensis* because of the frameshift mutation. For cloning and expression of unknown *cry* genes, pEG1064 was cleaved with *Bbu*I *and Asp*718 and the vector fragment purified following gel electrophoresis. Amplified fragments of unknown *cry* genes, obtained by PCR™ amplification of total *B. thuringiensis* DNA, were digested with the restriction endonucleases *Bbu*I *and Asp*718 and ligated into the *Bbu*I *and Asp*718 sites of the pEG1064 vector fragment. The ligation mixture was used to transform the Cry$^-$ *B. thuringiensis* strains, EG10368 or EG10650, to chloramphenicol resistance using an electroporation protocol previously described (Mettus and Macaluso, 1990) Chloramphenicol-resistant (Cm^R) isolates were evaluated for crystal protein production by phase-contrast microscopy. Crystal forming (Cry+) isolates were subsequently grown in C2 liquid broth medium (Donovan *et al.*, 1988) to obtain crystal protein for SDS-PAGE analysis and insect bioassay.

Because of the frameshift mutation within the *cry1Ac* gene, the crystal proteins obtained from the transformants could not be derived from the vector pEG1064. The Cry$^+$ transformants thus contained unknown *cry* gene fragments fused, at the *Asp*718 site, to a 3'-portion of the *cry1Ac* gene. Transcription of these gene fusions in *B. thuringiensis* was presumably directed from the σ^E promoter incorporated into the amplified *cry* gene fragment. The fusion proteins, containing the entire active toxin region of the unknown Cry protein, were capable of producing crystals in *B. thuringiensis*.

6.7 Example 7 -- Cloning of cry9-Related Genes

Total DNA was isolated from *B. thuringiensis* strain EG9290 for cloning studies. EG9290 was grown overnight at 30°C in 1X brain heart infusion, 0.5% glycerol (BHIG). In the morning, 500 µl of the overnight growth was suspended in 50 ml BHIG and the culture incubated at 30°C with agitation until the culture reached a Klett reading of 150 (red filter). The cells were harvested by centrifugation, suspended in 5 ml 1X GTE buffer containing 4 mg/ml lysozyme and 100 µg/ml Rnase A, and incubated at 37°C for 20 min. The cells were lysed by the addition of 0.5 ml of 20% SDS. The released DNA was precipitated by the addition of 2.5 ml 7.5 M ammonium acetate and 7 ml of isopropanol. The precipitated DNA was spooled out of

the mixture using a glass micropipette and washed in 80% ethanol. The DNA was resuspended in 10 ml 1X TE, extracted with one volume each of buffered phenol and chloroform:isoamyl alcohol (24:1), and precipitated as before. The spooled DNA was washed in 80% ethanol, allowed to air dry for several min, and suspended in 600 µl 1X TE. The DNA concentration was estimated at 500 µg/ml.

A library of EG9290 total DNA was constructed using partially digested *Mbo*I fragments of EG9290 DNA and the general methods described herein. The partial *Mbo*I fragments were inserted into the unique *Bam*HI site of cloning vector pHT315. The ligation mixture was used to transform *E. coli* Sure™ cells to ampicillin resistance by electroporation employing electrocompetent cells and protocols provided by Stratagene (La Jolla, CA) and the BioRad Gene Pulser™ apparatus (Bio-Rad Laboratories, Hercules, CA). Recombinant clones harboring *cry9*-type genes were identified by colony blot hybridization using a ^{32}P-labeled probe consisting of the putative *cry9C* fragment generated by amplification of EG9290 DNA with primers pr58 and pr59. Plasmid DNAs were extracted from the *E. coli* clones using a standard alkaline lysis procedure.

Plasmid DNAs from the *E. coli* recombinant clones were used to transform *B. thuringiensis* strain EG10368 to erythromycin resistance using the electroporation procedure described by Mettus and Macaluso (1990). Cells were plated onto starch agar plates containing 20 µg/ml erythromycin and incubated at 30°C. After six days, colonies with a more opaque appearance were recovered from the plates and streaked out onto fresh starch agar plates containing 20 µg/ml erythromycin to isolate single colonies. Colonies exhibiting a more opaque appearance were observed to produce large parasporal inclusions/crystals by phase-contrast microscopy.

Recombinant EG10368 clones producing parasporal inclusion/crystals were evaluated for crystal protein production in broth culture. Single colonies were inoculated into C2 medium containing 10 µg/ml erythromycin and grown at 30°C for 3 days at 28-30°C, at which time the cultures were fully sporulated and lysed. Spores and crystals were pelleted by centrifugation and resuspended in 20 mM Tris-HCl, 1 mM EDTA, pH 7.0. Aliquots of this material were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Two EG10368 recombinant clones, initially identified as 9290-2 and 9290-3, were observed to produce distinct proteins of ~130

kDa. 9290-2 was designated EG12102 and 9290-3 was designated EG12103. The EG12102 protein was designated CryET59 while the EG12103 protein was designated CryET60.

Plasmid DNAs were prepared from EG12102 and EG12103 using a standard alkaline lysis procedure. Digestion of the plasmids with the restriction endonuclease *Xba*I confirmed that the two strains harbored distinct *cry* genes. The *cry* plasmids of EG12102 and EG12103, designated pEG945 and pEG946, respectively, were used to transform *E. coli* Sure™ cells to ampicillin resistance by electroporation, employing electrocompetent cells and protocols provided by Stratagene Inc. The *E. coli* recombinant strain containing pEG945 was designated EG12132, and the *E. coli* recombinant strain containing pEG946 was designated EG12133. pEG945 and pEG946 were purified from the *E. coli* recombinant strains using the QIAGEN midi-column plasmid purification kit and protocols (QIAGEN Inc., Valencia, CA).

The *cryET83* gene was cloned from *B. thuringiensis* strain EG6346 subspecies *aizawai* using similar methods. Southern blot analysis of genomic DNA from EG6346 revealed a unique restriction fragment that hybridized to the *cryET59* probe. A series of degenerate oligonucleotide primers, pr95, pr97, and pr98, were designed to amplify *cry9*-related sequences from genomic DNA. The sequences of these primers are as shown:

pr95: 5'- GTWTGGACSCRTCGHGATGTGG -3' (SEQ ID NO:57)

pr97: 5'- TAATTTCTGCTAGCCCWATTTCTGGATTTAATTGTTGATC -3'

(SEQ ID NO:58)

pr98: 5'- ATWACNCAAMTWCCDTTRG -3' (SEQ ID NO:59)

where D = A, G; H = A, C, T; M =A, C; N = A, C, G, T; R = A, G; S = C, G; and W = A, T.

A PCR™ using Taq polymerase, Taq Extender™ (Stratagene, La Jolla, CA), the opposing primers pr95 and pr97, and total EG6346 DNA yielded a DNA fragment that was faintly visible on an ethidium bromide-stained agarose gel. This DNA served as the template for a second round of PCR™ using the opposing primers pr97 and pr98. The resulting amplified DNA fragment was suitable for cloning and served as a hybridization probe for subsequent cloning experiments. A library of EG6346 total DNA was constructed using partially digested 9-12 kb *Mbo*I fragments of EG6346 DNA ligated into the unique *Bam*HI site of cloning vector pHT315. *E. coli* recombinant clones harboring the *cryET83* gene were identified by colony blot hybridization using the EG6346-specific DNA fragment as a chemiluminescent hybridization

probe and the CDP-Star™ nucleic acid chemiluminescent reagent kit from NEN™ Life Science Products (Boston, MA) to prepare the hybridization probe. The recombinant plasmid harboring the *cryET83* gene was designated pEG397. The *E. coli* recombinant stain containing pEG397 was designated EG11786. The *B. thuringiensis* recombinant strain containing pEG397 was designated EG11785.

6.8 Example 8 -- Sequencing of Cloned B. thuringiensis Toxin Genes

Partial sequences for the cloned toxin genes were determined following established dideoxy chain-termination DNA sequencing procedures (Sanger *et al.*, 1977). Preparation of the double stranded plasmid template DNA was accomplished using a standard alkaline lysis procedure or using a QIAGEN plasmid purification kit (QIAGEN Inc., Valencia, CA). The sequencing reactions were performed using the Sequenase™ Version 2.0 DNA Sequencing Kit (United States Biochemical/Amersham Life Science Inc., Cleveland, OH) following the manufacturer's procedures and using ^{35}S-dATP as the labeling isotope (obtained from DuPont NEN® Research Products, Boston, MA). Denaturing gel electrophoresis of the reactions is done on a 6% (wt./vol.) acrylamide, 42% (wt./vol.) urea sequencing gel. The dried gels are exposed to Kodak X-OMAT AR X-ray film (Eastman Kodak Company, Rochester, NY) overnight at room temperature. Alternatively, some *cry* genes were sequenced using automated sequencing methods. DNA samples were sequenced using the ABI PRISM™ DyeDeoxy sequencing chemistry kit (Applied Biosystems, Foster City, CA) according to the manufacturer's suggested protocol. The completed reactions were run on as ABI 377 automated DNA sequencer. DNA sequence data were analyzed using Sequencher™ v3.0 DNA analysis software (Gene Codes Corp., Ann Arbor, MI). Successive oligonucleotides to be used for priming sequencing reactions were designed from the sequencing data of the previous set of reactions.

The sequence determination for the *cryI*-related genes involved the use of the oligonucleotide probe wd207, described in Example 2, as the initial sequencing primer. This oligonucleotide anneals to a conserved region of *cryI* genes, but because of the inverted and reversed orientation of wd207, it generates sequence towards the 5′-end of the coding region allowing sequence of the variable region of the gene to be read. A typical sequencing run of 250-300 nucleotides was usually sufficient to determine the identity of the gene. If additional data were necessary, one or more additional oligonucleotides could be synthesized to continue the sequence until it could be determined if the sequence was unique. In cases where wd207 did

not function well as a primer, other oligonucleotides, designed to anneal to conserved regions of *cry1* genes, were used. One such oligonucleotide was the KpnR primer described herein above.

The sequencing of the cloned *cry2*-related genes followed the same general procedures as those described for the *cry1* genes, except that oligonucleotides specific for conserved regions in *cry2* genes were used as sequencing primers. The two primers used in these examples were wd268 and wd269, shown below.

Primer wd268 corresponds to *cry2Aa* nucleotides 579-597
5'-AATGCAGATGAATGGGG-3' (SEQ ID NO:60).
Primer wd269 corresponds to *cry2Aa* 1740-1757
5'-TGATAATGGAGCTCGTT-3' (SEQ ID NO:61)

The sequencing of *cryET59* and *cryET60* commenced with the use of primer pr56. The sequencing of *cryET83* commenced with the use of primer pr98. Successive oligonucleotides to be used for priming sequencing reactions were designed from the sequencing data of the previous set of reactions.

The derived sequences were compared to sequences of known *cry* genes using the FSTNSCAN program in the PC/GENE sequence analysis package (Intelligenetics, Mountain View, CA). This analysis permitted a preliminary classification of the cloned *cry* genes with respect to previously-known *cry* genes (Table 11).

TABLE 6 - HOMOLOGY COMPARISON OF DNA SEQUENCES[1]

Cloned Gene	DNA Sequence Identity
cryET31	90% identity with SEQ ID NO:4 of WO 98/40490
cryET40	99% identity with *cry1Aa*
cryET43	88% identity with *cry1Bd1*
cryET44	90% identity with *cry1Da/1Db*
cryET45	91% identity with *cry1Da/1Db*
cryET46	98% identity with *cry1Ga*
cryET47	99% identity with *cry1Ab*
cryET49	95% identity with *cry1Ja*
cryET51	85% identity with *cry1Ac*
cryET52	84% identity with *cry1Da/1Db*
cryET53	99% identity with SEQ ID NO:8 of US 5,723,758
cryET54	99.8% identity with *cry1Be*
cryET56	80% identity with *cry1Ac*
cryET57	98% identity with *cry1Da*
cryET59	95% identity with *cry9Ca*
cryET60	99.6% identity with *cry9Aa*
cryET61	97% identity with *cry1Ha*
cryET62	99% identity with *cry1Ad*
cryET63	93% identity with *cry1Ac*
cryET64	91% identity with SEQ ID NO:9 of US 5,723,758
cryET66	76% identity with *cry1Ga*
cryET67	99% identity with SEQ ID NO:10 of US 5,723,758
cryET72	98% identity with SEQ ID NO:4 of WO 98/40490
cryET73	99% identity with SEQ ID NO:6 of WO 98/40490
cryET83	

[1] Ktup value set at 2 for FSTNSCAN. The *cryET59* and *cryET60* sequences were compared using the FASTA program (Ktup=6) in the PC/GENE sequence analysis package.

6.9 Example 9 -- Expression of Cloned Toxin Genes in a B. *thuringiensis* Host

Plasmid DNA was isolated from *E. coli* colonies identified by hybridization to a gene-specific probe. The isolated plasmid was then introduced into a crystal protein-negative (Cry-) strain of *B. thuringiensis* using the electroporation protocol of Mettus and Macaluso (1990). Each of the cloning vectors used (see Table 5) has a gene to confer antibiotic resistance on the cells harboring that plasmid. *B. thuringiensis* transformants were selected by growth on agar plates containing 25 mg/ml erythromycin (pHT315) or 5 mg/ml chloramphenicol (pEG597 and pEG1064). Antibiotic-resistant colonies were then evaluated for crystal protein production by phase-contrast microscropy. Crystal producing colonies were then grown in C2 medium (Donovan *et al.*, 1988) to obtain cultures which were analyzed by SDS-PAGE and insect bioassay.

C2 cultures were inoculated with cells from Cry^+ colonies and grown for three days at 25-30°C in the presence of the appropriate antibiotic. During this time the culture grew to

stationary phase, sporulated and lysed, releasing the protein inclusions into the medium. The cultures are harvested by centrifugation, which pellets the spores and crystals. The pellets were washed in a solution of 0.005% Triton X-100®, 2 mM EDTA and centrifuged again. The washed pellets were resuspended at one-tenth the original volume in 0.005% Triton X-100®, 2 mM EDTA.

Crystal protein were solubilized from the spores-crystal suspension by incubating the suspension in a solubilization buffer [0.14 M Tris-HCl pH 8.0, 2% (wt./vol.) sodium dodecyl sulfate (SDS), 5% (vol./vol.) 2-mercaptoethanol, 10% (vol./vol.) glycerol, and 0.1% bromphenol blue] at 100°C for 5 min. The solubilized crystal proteins were size-fractionated by SDS-PAGE using a gel with an acrylamide concentration of 10%. After size fractionation the proteins were visualized by staining with Coomassie Brilliant Blue R-250.

The expected size for Cryl- and Cry9-related crystal proteins was approximately 130 kDa. The expected size for Cry2-related proteins was approximately 65 kDa.

6.10 Example 10 -- Insecticidal Activity of the Cloned B. *thuringiensis* Toxin Genes

B. thuringiensis recombinant strains producing individual cloned *cry* genes were grown in C2 medium until the cultures were fully sporulated and lysed. These C2 cultures were used to evaluate the insecticidal activity of the crystal proteins produced. Each culture was diluted with 0.005% Triton® X-100 to achieve the appropriate dilution for two-dose bioassay screens. Fifty microliters of each dilution were topically applied to 32 wells containing 1.0 ml artificial diet per well (surface area of 175 mm^2). A single lepidopteran larvae was placed in each of the treated wells and the tray was covered by a clear perforated mylar sheet. With the exception of the *P. xylostella* bioassays, that employed 3rd instar larvae, all the bioassays were performed with neonate larvae. Larval mortality was scored after 7 days of feeding at 28-30 °C and percent mortality was expressed as ratio of the number of dead larvae to the total number of larvae treated (Table 12). In some instances, severe stunting of larval growth was observed after 7 days, and the ratio of stunted/unstunted larva was also recorded. The bioassay results shown in Table 7 demonstrate that the crystal proteins produced by the recombinant *B. thuringiensis* strains do exhibit insecticidal activity and, furthermore,

Table 7A. Bioassay evaluations with ET crystal proteins

	Spodoptera exigua					*Spodoptera frugiperda*		
	250nl/well % mortality	2500nl/well % mortality	# stunted /# treated			250nl/well % mortality	2500nl/well % mortality	# stunted /# treated
Cry1Ac	0	5	4/32			16	53	1/32
ET31	5	12	17/32			9	6	4/32
ET40	0	5	0			3	3	0
ET43	0	8	0			3	3	2/32
ET44	0	2	0			6	0	1/32
ET45	0	0	0			0	0	1/32
ET46	0	12	0			0	6	0
ET47	19	49	11/32			31	81	6/32
ET49	0	8	0			0	3	0
ET51	0	0	0			0	0	0
ET52	0	0	0			3	3	0
ET53	0	0	0			3	0	0
ET54	0	66	3/32			6	34	9/32
ET56	0	0	0			0	6	0
ET57	2	15	18/32			3	94	0
ET59	0	0	0			0	3	0
ET60	0	0	0			0	3	0
ET61	2	5	2/32			0	3	0
ET62	2	59	12/32			0	13	0
ET63	0	12	5/32			3	0	0
ET64	0	0	0			3	6	0
ET66	0	12	1/32			3	0	1/31
ET67	29	90	0			13	61	0
ET72	0	0	0			3	94	5/31
ET73	0	2	0			0	0	0
Control	8	8	0			0	0	0

Table 7B. Bioassay evaluations with ET crystal proteins

	Plutella xylostella			*Ostrinia nubilalis*		
	250nl/well % mortality	2500nl/well % mortality	# stunted /# treated	250nl/well % mortality	2500nl/well % mortality	# stunted /# treated
Cry1Ac	100	100	0	100	100	0
ET31	0	2	0	100	100	0
ET40	0	68	0	0	0	2/32
ET43	5	100	0	46	100	0
ET44	0	0	0	0	0	3/32
ET45	0	0	0	0	0	4/32
ET46	0	8	0	0	0	0
ET47	100	100	0	100	100	0
ET49	0	5	0	0	0	0
ET51	0	0	0	0	0	0
ET52	2	43	0	0	14	16/32
ET53	8	97	0	4	46	5/32
ET54	14	100	0	25	89	1/32
ET56	0	0	0	0	0	0
ET57	0	97	0	0	7	0
ET59	100	100	0	96	100	0
ET60	100	100	0	100	96	0
ET61	0	11	0	0	0	2/32
ET62	97	100	0	100	100	0
ET63	100	100	0	100	100	0
ET64	40	100	0	68	100	0
ET66	100	100	0	86	100	0
ET67	87	100	0	0	79	1/32
ET72	0	0	0	0	0	0
ET73	2	2	0	93	100	0
Control	2	2	0	0	0	0

Table 7C. Bioassay evaluations with ET crystal proteins

	Heliothis virescens			*Helicoverpa zea*	
	250nl/well % mortality	2500nl/well % mortality	# stunted /# treated	250nl/well % mortality	2500nl/well % mortality
Cry1Ac	100	100	0	100	100
ET31	97	97	1/32	8	81
ET40	2	5	2/32	2	5
ET43	87	97	1/32	0	2
ET44	8	5	1/32	5	8
ET45	0	11	0	8	18
ET46	12	25	0	0	8
ET47	87	100	0	83	100
ET49	8	2	0	11	15
ET51	2	15	0	5	5
ET52	0	31	1/32	93	11
ET53	22	64	2/32	90	61
ET54	15	64	5/32	2	5
ET56	0	11	0	8	0
ET57	2	0	0	11	28
ET59	28	84	4/32	2	2
ET60	56	97	1/32	31	28
ET61	5	5	0	8	5
ET62	44	87	4/32	21	64
ET63	100	100	0	100	100
ET64	0	21	0	5	0
ET66	0	8	1/32	0	5
ET67	18	93	1/32	0	68
ET72	34	64	11/32	8	2
ET73	42	90	2/32	8	48
Control	5	5	0	5	5

Table 7D. Bioassay evaluations with ET crystal proteins

	Agrotis ipsilon			*Trichoplusia ni*		
	250nl/well % mortality	2500nl/well % mortality	# stunted /# treated	250nl/well % mortality	2500nl/well % mortality	# stunted /# treated
Cry1Ac	94	100		100	100	0
ET31	6	6		90	100	0
ET40	0	6		13	32	0
ET43	0	45		100	100	0
ET44	6	13		16	26	0
ET45	0	6		13	39	0
ET46	0	0		29	74	0
ET47	0	34		97	100	0
ET49	3	0		13	81	0
ET51	0	0		3	19	0
ET52	0	28		81	100	0
ET53	25	81		74	100	0
ET54	3	6		100	100	0
ET56	3	3		16	26	0
ET57	13	74		19	100	0
ET59	3	3		10	84	0
ET60	3	0		97	100	0
ET61	6	28		29	52	0
ET62	23	58		100	100	0
ET63	3	0		100	100	0
ET64	0	0		87	100	0
ET66	13	91		26	81	0
ET67	3	0		6	100	0
ET72	0	0		23	74	8/32
ET73	13	6		94	100	0
Control	0	0		3	3	0

that the crystal proteins exhibit differential activity towards the lepidopteran species tested.

Additional bioassays were performed with the crystal proteins designated CryET59, CryET60, CryET66, and CryET83. Crystal proteins produced in C2 medium were quantified by SDS-PAGE and densitometry using the method described by Brussock, S. M. and Currier, T. C., 1990, "Use of Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis to Quantify *Bacillus thuringiensis* δ-Endotoxins", in *Analytical Chemistry of Bacillus thuringiensis* (L. A. Hickle and W. L. Fitch, eds.), The American Chemical Society, pp. 78-87.

TABLE 8 - Bioassay Evaluation of CryET59 and CryET60

		Percent mortality[1]							
Toxin	Dose ng/well	AI	HV	HZ	ON	PX	rPX	SE	TN
Control[2]	-	2	6	0	0	2	0	2	0
CryET59	100	2	37	0	94	100	100	2	13
CryET59	500	11	80	3	100	100	100	0	63
CryET59	5000	62	100	6	100	100	100	71	100
CryET60	500	0	93	22	100	100	100	0	100
CryET60	5000	2	100	25	100	100	100	14	100

[1]AI = *Agrotis ipsilon*, HV = *Heliothis virescens*, HZ = *Helicoverpa zea*, ON = *Ostrinia nubilalis*, PX = *Plutella xylostella*, rPX = *Plutella xylostella* colony resistant to Cry1A and Cry IF toxins, SE = Spodoptera *exigua*, TN = *Trichoplusia ni*.

[2]Control = no toxin added.

The procedure was modified to eliminate the neutralization step with 3M HEPES. Crystal proteins resolved by SDS-PAGE were quantified by densitometry using a Molecular Dynamics model 300A computing densitometer and purified bovine serum albumin (Pierce, Rockford, IL) as a standard.

The bioassay results shown in Table 8 demonstrate that CryET59 and CryET60 are toxic to a number of lepidopteran species, including a colony of *P. xylostella* that is resistant to Cry1A and Cry1F crystal proteins. Eight-dose assays with CryET66 also demonstrated excellent toxicity towards both the susceptible and resistant colonies of *P. xylostella* (Table 14). In this instance, eight crystal protein concentrations were prepared by serial dilution of the crystal protein suspensions in 0.005% Triton® X-100 and 50 ul of each concentration was topically applied to wells containing 1.0 ml of artificial diet. After the wells had dried, a single larvae was placed in each of the treated wells and the tray was covered by a clear perforated mylar sheet (32 larvae for each crystal protein concentration). Larval mortality was scored after 7 days of feeding at 28-30 °C. Mortality data was expressed as LC_{50} and LC_{95} values, the concentration of crystal protein (ng/175 mm^2 diet well) causing 50% and 95% mortality, respectively (Daum, 1970).

Table 9: Toxicity of CryET66 towards *Plutella xylostella*

Toxin	LC_{50}[1]	95% C.I.	LC_{95}[2]	Slope
Cry1Ac	8.05	5.0-15.2	52.94	2.01
Cry1C	25.06	15.7-40.6	117.07	2.46
CryET66	0.42	0.4-0.5	1.4	3.13

Toxicity of CryET66 towards Cry1A-resistant *Plutella xylostella*

Toxin	LC_{50}[1]	95% C.I.	LC_{95}[2]	Slope
Cry1Ac	*No significant mortality >			
Cry1C	27.32	15.4-51.1	156.13	2.17
CryET66	1.65	1.3-2.0	6.41	2.79

[1] the concentration of crystal protein, in nanograms of crystal protein per well, required to achieve 50% mortality

[2] the concentration of crystal protein, in nanograms of crystal protein per well, required to achieve 95% mortality.

Table 15 shows that the CryET83 protein exhibits toxicity towards a wide variety of lepidopteran pests and may exhibit improved toxicity towards *S. exigua* and *H. virescens* when compared to the other Cry9-type proteins CryET59 and CryET60.

Table 10 - Toxicity of CryET83 towards lepidopteran larvae[1]

Dose[2]	AI[3]	HV	HZ	ON	PX	SE	SF	TN
5					5			
10				9				
50		53			75			69
100				91				
500	0	100				67		100
5000	32					100		
10000			84				100	

[1] Toxicity calculated as percent mortality among treated larvae.
[2] ng CryET83 crystal protein/175 mm^2 diet well
[3] Abbreviations described in Table 8; SF = *Spodoptera frugiperda*

The recombinant *B. thuringiensis* strains listed in Table 5 were deposited with the ARS Patent Culture Collection and had been assigned the NRRL deposit numbers shown in Table 11.

Table 11. Biological Deposits

Polypeptide Designation	Polypeptide Seq. ID No.:	Polynucleotide Seq ID No.:	Recomb. Strain	NRRL Deposit No.:
Cry ET31	2	1	EG11562	B-21921
Cry ET40	4	3	EG11901	B-21922
Cry ET43	6	5	EG7692	B-21923
Cry ET44	8	7	EG11629	B-21924
Cry ET45	10	9	EG7694	B-21925
Cry ET46	12	11	EG7695	B-21926
Cry ET47	14	13	EG7696	B-21927
Cry ET49	16	15	EG11630	B-21928
Cry ET51	18	17	EG11921	B-21929
Cry ET52	20	19	EG11584	B-21930
Cry ET53	22	21	EG11906	B-21931

Polypeptide Designation	Polypeptide Seq. ID No.:	Polynucleotide Seq ID No.:	Recomb. Strain	NRRL Deposit No.:
Cry ET54	63	62	EG11907	B-21932
Cry ET56	24	23	EG11909	B-21933
Cry ET57	26	25	EG11910	B-21934
Cry ET59	28	27	EG12102	B-21935
Cry ET60	30	29	EG12103	B-21936
Cry ET61	32	31	EG11634	B-21937
Cry ET62	34	33	EG11635	B-21938
Cry ET63	36	35	EG11636	B-21939
Cry ET64	38	37	EG11638	B-21940
Cry ET66	40	39	EG11640	B-21941
Cry ET67	42	41	EG11642	B-21942
Cry ET68	44	43	EG11644	B-30137
Cry ET72	46	45	EG11440	B-21943
Cry ET73	48	47	EG11465	B-21944
CryET83	50	49	EG11785	B-30138

6.11 Example 11 -- Modification of *cry* Genes for Expression in Plants

Wild-type *cry* genes are known to be expressed poorly in plants as a full length gene or as a truncated gene. Typically, the G+C content of a *cry* gene is low (37%) and often contains many A+T rich regions, potential polyadenylation sites and numerous ATTTA sequences. Table 12 shows a list of potential polyadenylation sequences which should be avoided when preparing the "plantized" gene construct.

Table 12 - LIST OF SEQUENCES OF POTENTIAL POLYADENYLATION SIGNALS

AATAAA*	AAGCAT
AATAAT*	ATTAAT
AACCAA	ATACAT
ATATAA	AAAATA
AATCAA	ATTAAA**
ATACTA	AATTAA**
ATAAAA	AATACA**
ATGAAA	CATAAA**

* indicates a potential major plant polyadenylation site.

** indicates a potential minor animal polyadenylation site.

All others are potential minor plant polyadenylation sites.

The regions for mutagenesis may be selected in the following manner. All regions of the DNA sequence of the *cry* gene are identified which contained five or more consecutive base pairs which were A or T. These were ranked in terms of length and highest percentage of A+T in the surrounding sequence over a 20-30 base pair region. The DNA is analysed for regions which might contain polyadenylation sites or ATTTA sequences. Oligonucleotides are then designed which maximize the elimination of A+T consecutive regions which contained one or more polyadenylation sites or ATTTA sequences. Two potential plant polyadenylation sites have been

shown to be more critical based on published reports. Codons are selected which increase G+C content, but do not generate restriction sites for enzymes useful for cloning and assembly of the modified gene (*e.g.*, *Bam*HI, *Bgl*II, *Sac*I, *Nco*I, *Eco*RV, *etc.*). Likewise condons are avoided which contain the doublets TA or GC which have been reported to be infrequently-found codons in plants.

Although the CaMV35S promoter is generally a high level constitutive promoter in most plant tissues, the expression level of genes driven the CaMV35S promoter is low in floral tissue relative to the levels seen in leaf tissue. Because the economically important targets damaged by some insects are the floral parts or derived from floral parts (*e.g.*, cotton squares and bolls, tobacco buds, tomato buds and fruit), it is often advantageous to increase the expression of crystal proteins in these tissues over that obtained with the CaMV35S promoter.

The 35S promoter of Figwort Mosaic Virus (FMV) is analogous to the CaMV35S promoter. This promoter has been isolated and engineered into a plant transformation vector. Relative to the CaMV promoter, the FMV 35S promoter is highly expressed in the floral tissue, while still providing similar high levels of gene expression in other tissues such as leaf. A plant transformation vector, may be constructed in which the full length synthetic *cry* gene is driven by the FMV 35S promoter. Tobacco plants may be transformed with the vector and compared for expression of the crystal protein by Western blot or ELISA immunoassay in leaf and floral tissue. The FMV promoter has been used to produce relatively high levels of crystal protein in floral tissue compared to the CaMV promoter.

6.12 Example 12 -- Expression of Synthetic *cry* Genes with ssRUBISCO Promoters and Chloroplast Transit Peptides

The genes in plants encoding the small subunit of RUBISCO (SSU) are often highly expressed, light regulated and sometimes show tissue specificity. These expression properties are largely due to the promoter sequences of these genes. It has been possible to use SSU promoters to express heterologous genes in transformed plants. Typically a plant will contain multiple SSU genes, and the expression levels and tissue specificity of different SSU genes will be different. The SSU proteins are encoded in the nucleus and synthesized in the cytoplasm as precursors that contain an N-terminal extension known as the chloroplast transit peptide (CTP). The CTP directs the precursor to the chloroplast and promotes the uptake of the SSU protein into

the chloroplast. In this process, the CTP is cleaved from the SSU protein. These CTP sequences have been used to direct heterologous proteins into chloroplasts of transformed plants.

The SSU promoters might have several advantages for expression of heterologous genes in plants. Some SSU promoters are very highly expressed and could give rise to expression levels as high or higher than those observed with the CaMV35S promoter. The tissue distribution of expression from SSU promoters is different from that of the CaMV35S promoter, so for control of some insect pests, it may be advantageous to direct the expression of crystal proteins to those cells in which SSU is most highly expressed. For example, although relatively constitutive, in the leaf the CaMV35S promoter is more highly expressed in vascular tissue than in some other parts of the leaf, while most SSU promoters are most highly expressed in the mesophyll cells of the leaf. Some SSU promoters also are more highly tissue specific, so it could be possible to utilize a specific SSU promoter to express the protein of the present invention in only a subset of plant tissues, if for example expression of such a protein in certain cells was found to be deleterious to those cells. For example, for control of Colorado potato beetle in potato, it may be advantageous to use SSU promoters to direct crystal protein expression to the leaves but not to the edible tubers.

Utilizing SSU CTP sequences to localize crystal proteins to the chloroplast might also be advantageous. Localization of the *B. thuringiensis* crystal proteins to the chloroplast could protect these from proteases found in the cytoplasm. This could stabilize the proteins and lead to higher levels of accumulation of active toxin. *cry* genes containing the CTP may be used in combination with the SSU promoter or with other promoters such as CaMV35S.

6.13 Example 13 -- Targeting of Cry Proteins to the Extracellular Space or Vacuole through the Use of Signal Peptides

The *B. thuringiensis* proteins produced from the synthetic genes described here are localized to the cytoplasm of the plant cell, and this cytoplasmic localization results in plants that are insecticidally effective. It may be advantageous for some purposes to direct the *B. thuringiensis* proteins to other compartments of the plant cell. Localizing *B. thuringiensis* proteins in compartments other than the cytoplasm may result in less exposure of the *B. thuringiensis* proteins to cytoplasmic proteases leading to greater accumulation of the protein yielding enhanced insecticidal activity. Extracellular localization could lead to more efficient exposure of certain insects to the *B. thuringiensis* proteins leading to greater efficacy. If a

B. thuringiensis protein were found to be deleterious to plant cell function, then localization to a noncytoplasmic compartment could protect these cells from the protein.

In plants as well as other eukaryotes, proteins that are destined to be localized either extracellularly or in several specific compartments are typically synthesized with an N-terminal amino acid extension known as the signal peptide. This signal peptide directs the protein to enter the compartmentalization pathway, and it is typically cleaved from the mature protein as an early step in compartmentalization. For an extracellular protein, the secretory pathway typically involves cotranslational insertion into the endoplasmic reticulum with cleavage of the signal peptide occurring at this stage. The mature protein then passes through the Golgi body into vesicles that fuse with the plasma membrane thus releasing the protein into the extracellular space. Proteins destined for other compartments follow a similar pathway. For example, proteins that are destined for the endoplasmic reticulum or the Golgi body follow this scheme, but they are specifically retained in the appropriate compartment. In plants, some proteins are also targeted to the vacuole, another membrane bound compartment in the cytoplasm of many plant cells. Vacuole targeted proteins diverge from the above pathway at the Golgi body where they enter vesicles that fuse with the vacuole.

A common feature of this protein targeting is the signal peptide that initiates the compartmentalization process. Fusing a signal peptide to a protein will in many cases lead to the targeting of that protein to the endoplasmic reticulum. The efficiency of this step may depend on the sequence of the mature protein itself as well. The signals that direct a protein to a specific compartment rather than to the extracellular space are not as clearly defined. It appears that many of the signals that direct the protein to specific compartments are contained within the amino acid sequence of the mature protein. This has been shown for some vacuole targeted proteins, but it is not yet possible to define these sequences precisely. It appears that secretion into the extracellular space is the "default" pathway for a protein that contains a signal sequence but no other compartmentalization signals. Thus, a strategy to direct *B. thuringiensis* proteins out of the cytoplasm is to fuse the genes for synthetic *B. thuringiensis* genes to DNA sequences encoding known plant signal peptides. These fusion genes will give rise to *B. thuringiensis* proteins that enter the secretory pathway, and lead to extracellular secretion or targeting to the vacuole or other compartments.

Signal sequences for several plant genes have been described. One such sequence is for the tobacco pathogenesis related protein PR1b has been previously described (Cornelissen *et al.*, 1986). The PR1b protein is normally localized to the extracellular space. Another type of signal peptide is contained on seed storage proteins of legumes. These proteins are localized to the protein body of seeds, which is a vacuole like compartment found in seeds. A signal peptide DNA sequence for the β-subunit of the 7S storage protein of common bean (*Phaseolus vulgaris*), PvuB has been described (Doyle *et al.*, 1986). Based on the published these published sequences, genes may be synthesized chemically using oligonucleotides that encode the signal peptides for PR1b and PvuB. In some cases to achieve secretion or compartmentalization of heterologous proteins, it may be necessary to include some amino acid sequence beyond the normal cleavage site of the signal peptide. This may be necessary to insure proper cleavage of the signal peptide.

6.14 Example 14 -- Isolation of Transgenic Plants Resistant to Insects Using *cry* Transgenes

6.64.1 Plant Gene Construction

The expression of a plant gene which exists in double-stranded DNA form involves transcription of messenger RNA (mRNA) from one strand of the DNA by RNA polymerase enzyme, and the subsequent processing of the mRNA primary transcript inside the nucleus. This processing involves a 3′ non-translated region which adds polyadenylate nucleotides to the 3′ end of the RNA. Transcription of DNA into mRNA is regulated by a region of DNA usually referred to as the "promoter". The promoter region contains a sequence of bases that signals RNA polymerase to associate with the DNA and to initiate the transcription of mRNA using one of the DNA strands as a template to make a corresponding strand of RNA.

A number of promoters which are active in plant cells have been described in the literature. Such promoters may be obtained from plants or plant viruses and include, but are not limited to, the nopaline synthase (NOS) and octopine synthase (OCS) promoters (which are carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*), the cauliflower mosaic virus (CaMV) 19S and 35S promoters, the light-inducible promoter from the small subunit of ribulose 1,5-bisphosphate carboxylase (ssRUBISCO, a very abundant plant polypeptide), and the Figwort Mosaic Virus (FMV) 35S promoter. All of these promoters have been used to create various

types of DNA constructs which have been expressed in plants (see *e.g.*, U. S. Patent No. 5,463,175, specifically incorporated herein by reference).

The particular promoter selected should be capable of causing sufficient expression of the enzyme coding sequence to result in the production of an effective amount of protein. One set of preferred promoters are constitutive promoters such as the CaMV35S or FMV35S promoters that yield high levels of expression in most plant organs (U. S. Patent No. 5,378,619, specifically incorporated herein by reference). Another set of preferred promoters are root enhanced or specific promoters such as the CaMV derived 4 as-1 promoter or the wheat POX1 promoter (U. S. Patent No. 5,023,179, specifically incorporated herein by reference; Hertig *et al.*, 1991). The root enhanced or specific promoters would be particularly preferred for the control of corn rootworm (*Diabroticus* spp.) in transgenic corn plants.

The promoters used in the DNA constructs (*i.e.* chimeric plant genes) of the present invention may be modified, if desired, to affect their control characteristics. For example, the CaMV35S promoter may be ligated to the portion of the ssRUBISCO gene that represses the expression of ssRUBISCO in the absence of light, to create a promoter which is active in leaves but not in roots. The resulting chimeric promoter may be used as described herein. For purposes of this description, the phrase "CaMV35S" promoter thus includes variations of CaMV35S promoter, *e.g.*, promoters derived by means of ligation with operator regions, random or controlled mutagenesis, *etc.* Furthermore, the promoters may be altered to contain multiple "enhancer sequences" to assist in elevating gene expression.

The RNA produced by a DNA construct of the present invention also contains a 5′ non-translated leader sequence. This sequence can be derived from the promoter selected to express the gene, and can be specifically modified so as to increase translation of the mRNA. The 5′ non-translated regions can also be obtained from viral RNA's, from suitable eucaryotic genes, or from a synthetic gene sequence. The present invention is not limited to constructs wherein the non-translated region is derived from the 5′ non-translated sequence that accompanies the promoter sequence.

For optimized expression in monocotyledenous plants such as maize, an intron should also be included in the DNA expression construct. This intron would typically be placed near the 5′ end of the mRNA in untranslated sequence. This intron could be obtained from, but not limited to, a set of introns consisting of the maize *hsp70* intron (U. S. Patent No. 5,424,412;

specifically incorporated herein by reference) or the rice *Act1* intron (McElroy *et al.*, 1990). As shown below, the maize *hsp70* intron is useful in the present invention.

As noted above, the 3′ non-translated region of the chimeric plant genes of the present invention contains a polyadenylation signal which functions in plants to cause the addition of adenylate nucleotides to the 3′ end of the RNA. Examples of preferred 3′ regions are (1) the 3′ transcribed, non-translated regions containing the polyadenylate signal of *Agrobacterium* tumor-inducing (Ti) plasmid genes, such as the nopaline synthase (NOS) gene and (2) plant genes such as the pea ssRUBISCO E9 gene (Fischhoff *et al.*, 1987).

6.14.2 Plant Transformation and Expression

A plant gene containing a structural coding sequence of the present invention can be inserted into the genome of a plant by any suitable method. Suitable plant transformation vectors include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed, *e.g.*, by Herrera-Estrella (1983), Bevan (1983), Klee (1985) and Eur. Pat. Appl. Publ. No. EP0120516. In addition to plant transformation vectors derived from the Ti or root-inducing (Ri) plasmids of *Agrobacterium*, alternative methods can be used to insert the DNA constructs of this invention into plant cells. Such methods may involve, for example, the use of liposomes, electroporation, chemicals that increase free DNA uptake, free DNA delivery *via* microprojectile bombardment, and transformation using viruses or pollen (Fromm *et al.*, 1986; Armstrong *et al.*, 1990; Fromm *et al.*, 1990).

6.14.3 Construction of Monocot Plant Expression Vectors for *cry* Genes

For efficient expression of *cry* genes in transgenic plants, the gene must have a suitable sequence composition (Diehn *et al.*, 1996). To place the *cry* gene in a vector suitable for expression in monocotyledonous plants (*i.e.* under control of the enhanced Cauliflower Mosaic Virus 35S promoter and link to the *hsp70* intron followed by a nopaline synthase polyadenylation site as in U. S. Patent No. 5,424,412, specifically incorporated herein by reference), a vector such as pMON19469 may be used. Such a vector is conveniently digested with *Nco*I and *Eco*RI restriction enzymes. The larger vector band of approximately 4.6 kb is then electrophoresed, purified, and ligated with T4 DNA ligase to an *Nco*I-*Eco*RI fragment which contains the synthetic *cry* gene. The ligation mix is then transformed into *E. coli*, carbenicillin resistant colonies recovered and plasmid DNA recovered by DNA miniprep

procedures. The DNA is then subjected to restriction endonuclease analysis with enzymes such as *Nco*I and *Eco*RI (together), *Not*I, and/or *Pst*I individually or in combination, to identify clones containing the *cry* coding sequence fused to an intron such as the *hsp70* intron, placed under the control of the enhanced CaMV35S promoter.

To place the gene in a vector suitable for recovery of stably transformed and insect resistant plants, the 3.75-kb *Not*I restriction fragment from pMON33708 containing the lysine oxidase coding sequence fused to the *hsp70* intron under control of the enhanced CaMV35S promoter may be isolated by gel electrophoresis and purification. This fragment is then ligated with a vector such as pMON30460 which has been previously treated with *Not*I and calf intestinal alkaline phosphatase (pMON30460 contains the neomycin phosphotransferase coding sequence under control of the CaMV35S promoter). Kanamycin resistant colonies may then be obtained by transformation of this ligation mix into *E. coli* and colonies containing the desired plasmid may be identified by restriction endonuclease digestion of plasmid miniprep DNAs. Restriction enzymes such as *Not*I, *Eco*RV, *Hin*dIII, *Nco*I, *Eco*RI, and *Bgl*II may be used to identify the appropriate clones in which the orientation of both genes are in tandem (*i.e.* the 3′ end of the *cry* expression cassette is linked to the 5′ end of the *nptII* expression cassette). Expression of the Cry protein by the resulting plasmid in corn protoplasts may be confirmed by electroporation of the vector DNA into protoplasts followed by protein blot and ELISA analysis. This vector may be introduced into the genomic DNA of corn embryos by particle gun bombardment followed by paromomycin selection to obtain corn plants expressing the *cry* gene essentially as described in U. S. Patent No. 5,424,412, specifically incorporated herein by reference.

As an example, the vector may be introduced *via* cobombardment with a hygromycin resistance conferring plasmid into immature embryo scutella (IES) of maize, followed by hygromycin selection, and regeneration. Transgenic corn lines expressing the *cry* protein may then be identified by ELISA analysis. Progeny seed from these events may then be subsequently tested for protection from insect feeding.

7.0 References

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All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims. Accordingly, the exclusive rights sought to be patented are as described in the claims below.

CLAIMS:

1. An isolated polypeptide at least 85% identical to SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:40, or SEQ ID NO:44.

2. An isolated polypeptide at least 91% identical to SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:38, SEQ ID NO:40, or SEQ ID NO:44.

3. An isolated polypeptide at least 95% identical to SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:28, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:44, or SEQ ID NO:50.

4. An isolated polypeptide at least 99% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50 or SEQ ID NO: 63.

5. The polypeptide of claim 4, comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50 and SEQ ID NO: 63.

6. An isolated nucleic acid sequence encoding the polypeptide of any preceding claim.

7. A composition comprising the polypeptide of any of claims 1 to 5, and a diluent.

8. The composition of claim 7, wherein the polypeptide is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID

NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50 and SEQ ID NO: 63.

9. The composition of claim 7, comprising a cell extract, cell suspension, cell homogenate, cell lysate, cell supernatant, cell filtrate, or cell pellet of *Bacillus thuringiensis* cells.

10. The composition of claim 7, wherein said composition is a powder, dust, pellet, granule, spray, emulsion, colloid, or solution.

11. The composition of claim 7, comprising from about 1% to about 99% by weight of said polypeptide.

12. An insecticidal polypeptide prepared by a process comprising the steps of:

(a) culturing a *Bacillus thuringiensis* cell having the accession number NRRL B-21784, NRRL B-21783, NRRL B-21917, NRRL B-21786, NRRL B-21787, NRRL B-21785, NRRL B-21788, NRRL B-21915 or NRRL B-21916 under conditions effective to produce an insecticidal polypeptide; and

(b) obtaining from said cell the insecticidal polypeptide so produced.

13. A *Bacillus thuringiensis* cell having the NRRL accession number NRRL B-21784, NRRL B-21783, NRRL B-21917, NRRL B-21786, NRRL B-21787, NRRL B-21785, NRRL B-21788, NRRL B-21915 or NRRL B-21916.

14. An isolated polynucleotide at least 85% identical to SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:39, or SEQ ID NO:43.

15. The polynucleotide of claim 15, wherein the polynucleotide is at least 95% identical to SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:43, or SEQ ID NO:49.

16. The polynucleotide of claim 15, wherein the polynucleotide is at least 99% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID

NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, or SEQ ID NO:62.

17. The polynucleotide of claim 15, comprising the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, or SEQ ID NO:62.

18. The polynucleotide of any of claims 14-17, wherein the isolated polynucleotide is provided in a vector.

19. The polynucleotide of any of claims 14-17, wherein the isolated polynucleotide is operably linked to a promoter.

20. The polynucleotide of claim 19, wherein the promoter is a plant-expressible promoter.

21. The polynucleotide of claim 20, wherein the plant-expressible promoter is selected from the group consisting of corn sucrose synthetase 1, corn alcohol dehydrogenase 1, corn light harvesting complex, corn heat shock protein, pea small subunit RuBP carboxylase, Ti plasmid mannopine synthase, Ti plasmid nopaline synthase, petunia chalcone isomerase, bean glycine rich protein 1, Potato patatin, lectin, CaMV 35S, and the S-E9 small subunit RuBP carboxylase promoter.

22. The polynucleotide of claim 18, wherein the vector is a plasmid, baculovirus, artificial chromosome, virion, cosmid, phagemid, phage or viral vector.

23. A transformed host cell comprising a nucleic acid sequence encoding the polypeptide of any of claims 1 to 5.

24. The transformed host cell of claim 23, wherein the nucleic acid is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID

NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, and SEQ ID NO:62.

25. The transformed host cell of claim 23, further defined as a prokaryotic or eukaryotic host cell.

26. The transformed host cell of claim 23, further defined as a bacterial cell or a plant cell.

27. The transformed host cell of claim 26, wherein said bacterial cell is a *Bacillus thuringiensis*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus cereus*, *Escherichia*, *Salmonella*, *Agrobacterium* or *Pseudomonas* cell.

28. The transformed host cell of claim 26, wherein said bacterial cell is a *Bacillus thuringiensis* NRRL B-21784, NRRL B-21783, NRRL B-21917, NRRL B-21786, NRRL B-21787, NRRL B-21785, NRRL B-21788, NRRL B-21915 or NRRL B-21916 cell.

29. The transformed host cell of claim 27, wherein said bacterial cell is an *Agrobacterium tumefaciens* cell.

30. The transformed host cell of claim 26, further defined as a monocotyledonous or dicotyledonous plant cell.

31. The transformed host cell of claim 30, wherein said plant cell is selected from the group consisting of a corn, wheat, soybean, oat, cotton, rice, rye, sorghum, sugarcane, tomato, tobacco, kapok, flax, potato, barley, turf grass, pasture grass, berry, fruit, legume, vegetable, ornamental plant, shrub, cactus, succulent, and tree cell.

32. The transformed host cell of claim 30, wherein said plant cell is a corn, wheat, rice, or sugarcane cell.

33. The transformed host cell of claim 30, wherein said plant cell is a soybean, cotton, potato, tomato, or tobacco cell.

34. A transgenic plant having incorporated into its genome a selected polynucleotide comprising a first sequence region that encodes the polypeptide of any of claims 1 to 5.

35. The transgenic plant of claim 34, wherein said first sequence region encodes SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID

NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, or SEQ ID NO: 63.

36. The transgenic plant of claim 34, wherein said first sequence region comprises SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, or SEQ ID NO:62.

37. The transgenic plant of claim 34, further defined as a monocotyledonous plant.

38. The transgenic plant of claim 34, further defined as a corn, wheat, oat, rice, barley, turf grass, or pasture grass plant.

39. The transgenic plant of claim 34, further defined as a dicotyledonous plant.

40. The transgenic plant of claim 34, further defined as a legume, soybean, tobacco, tomato, potato, cotton, fruit, berry, vegetable or tree.

41. A progeny of any generation of the transgenic plant of claim 34, wherein said progeny comprises said first selected sequence region.

42. A seed of any generation of the plant of claim 34, wherein said seed comprises said first sequence region.

43. A seed of any generation of the progeny of claim 39, wherein said seed comprises said first sequence region.

44. A plant of any generation of the seed of claim 42 or 43, wherein said plant comprises said first sequence region.

45. A method for controlling Lepdopteran insects comprising contacting said insect with the polypeptide of any of claims 1 to 5.

46. The method of claim 45, wherein the polypeptide is provided in a powder, dust, pellet, granule, spray, emulsion, colloid, or solution.

47. The method of claim 45, wherein the polypeptide is provided in a transformed host cell.

48. The method of claim 47, wherein the transformed host cell is a bacterial or plant cell.

49. The method of claim 45, wherein the polypeptide is provided in a transgenic plant.

50. The method of claim 49, wherein the plant is a corn, cotton, or soybean plant.

51. A method of preparing an insect resistant plant comprising:

(a) contacting recipient plant cells with a polynucleotide composition comprising at least a first nucleic acid sequence encoding the polypeptide of any of claims 1 to 5;

(b) selecting a recipient plant cell comprising the first nucleic acid sequence; and

(c) regenerating a plant from the selected cell;

wherein said plant has enhanced insect resistance relative to the corresponding non-transformed plant.

8.0 Sequence Listing

SEQUENCE LISTING

<110> Baum, James A.
Chu, Chih-Rei
Donovan, William P.
Gilmer, Amy J.
Rupar, Mark J.

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465                 470                 475                 480

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Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe Thr Ile Ser
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    290                 295                 300
Asn Tyr Val Leu Asn Gly Phe Ser Gly Ala Arg Leu Thr Gln Thr Phe
305                 310                 315                 320
Pro Asn Ile Gly Gly Leu Pro Gly Thr Thr Thr Thr His Ala Leu Leu
                325                 330                 335
Ala Ala Arg Val Asn Tyr Ser Gly Gly Val Ser Ser Gly Asp Ile Gly
            340                 345                 350
Ala Val Phe Asn Gln Asn Phe Ser Cys Ser Thr Phe Leu Pro Pro Leu
        355                 360                 365
Leu Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Ser Asp Arg Gly
    370                 375                 380
Gly Val Asn Thr Val Thr Asn Trp Gln Thr Glu Ser Phe Glu Ser Thr
385                 390                 395                 400
Leu Gly Leu Arg Cys Gly Ala Phe Thr Ala Arg Gly Asn Ser Asn Tyr
                405                 410                 415
Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro Leu Val Val
            420                 425                 430
Arg Asn Glu Asp Leu Arg Arg Pro Leu His Tyr Asn Glu Ile Arg Asn
        435                 440                 445
Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Leu Arg Ala Tyr Met Val
    450                 455                 460
Ser Val His Asn Arg Lys Asn Asn Ile Tyr Ala Val His Glu Asn Gly
465                 470                 475                 480
Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe Thr Ile Ser
                485                 490                 495
Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe Ile Ser
            500                 505                 510
Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln Ser Asn
        515                 520                 525
Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser Tyr Asn Leu
    530                 535                 540
Tyr Leu Arg Val Ser Ser Leu Gly Asn Ser Thr Ile Arg Val Thr Ile
545                 550                 555                 560
Asn Gly Arg Val Tyr Thr Ala Ser Asn Val Asn Thr Thr Thr Asn Asn
                565                 570                 575
Asp Gly Val Asn Asp Asn Gly Ala Arg Phe Leu Asp Ile Asn Met Gly
            580                 585                 590
Asn Val Val Ala Ser Asp Asn Thr Asn Val Pro Leu Asp Ile Asn Val
        595                 600                 605
Thr Phe Asn Ser Gly Thr Gln Phe Glu Leu Met Asn Ile Met Phe Val
    610                 615                 620
Pro Thr Asn Leu Pro Pro Ile Tyr
625                 630
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<210> 3
<211> 729
<212> DNA

<213> Bacillus thuringiensis

<400> 3
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cgtattcaat tcaatgacat gaacagtgcc cttacaaccg ctattcctct tttggcagtt 180
caaaattatc aagttcctct tttatcagta tatgttcaag ctgcaaattt acatttatca 240
gttttgagag atgtttcagt gtttggacaa aggtggggat ttgatgccgc gactatcaat 300
agtcgttata atgatttaac taggcttatt ggcaactata cagattatgc tgtgcgctgg 360
tacaatacgg gattagagcg tgtatgggga ccggattcta gagattgggt aaggtataat 420
caatttagaa gagagctaac acttactgta ttagatatcg ttgctctatt ctcaaattat 480
gatagtcgaa ggtatccaat tcgaacagtt tcccaattaa caagagaaat ttatacgaac 540
ccagtattag aaaattttga tggtagtttt cgtggaatgg ctcagagaat agaacagaat 600
attaggcaac cacatcttat ggatatcctt aatagtataa ccatttatac tgatgtgcat 660
agaggcttta attattggtc agggcatcaa ataacagctt ctcctgtagg gttttcagga 720
ccagaattc 729

<210> 4
<211> 243
<212> PRT
<213> Bacillus thuringiensis

<400> 4

Phe	Ala	Arg	Asn	Gln	Ala	Ile	Ser	Arg	Leu	Glu	Gly	Leu	Ser	Asn	Leu
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Tyr	Gln	Ile	Tyr	Ala	Glu	Ser	Phe	Arg	Glu	Trp	Glu	Ala	Asp	Pro	Thr
			20					25					30		
Asn	Pro	Ala	Leu	Arg	Glu	Glu	Met	Arg	Ile	Gln	Phe	Asn	Asp	Met	Asn
		35					40					45			
Ser	Ala	Leu	Thr	Thr	Ala	Ile	Pro	Leu	Leu	Ala	Val	Gln	Asn	Tyr	Gln
	50					55					60				
Val	Pro	Leu	Leu	Ser	Val	Tyr	Val	Gln	Ala	Ala	Asn	Leu	His	Leu	Ser
65					70					75					80
Val	Leu	Arg	Asp	Val	Ser	Val	Phe	Gly	Gln	Arg	Trp	Gly	Phe	Asp	Ala
				85					90					95	
Ala	Thr	Ile	Asn	Ser	Arg	Tyr	Asn	Asp	Leu	Thr	Arg	Leu	Ile	Gly	Asn
			100					105					110		
Tyr	Thr	Asp	Tyr	Ala	Val	Arg	Trp	Tyr	Asn	Thr	Gly	Leu	Glu	Arg	Val
		115					120					125			
Trp	Gly	Pro	Asp	Ser	Arg	Asp	Trp	Val	Arg	Tyr	Asn	Gln	Phe	Arg	Arg
	130					135					140				
Glu	Leu	Thr	Leu	Thr	Val	Leu	Asp	Ile	Val	Ala	Leu	Phe	Ser	Asn	Tyr
145					150					155					160
Asp	Ser	Arg	Arg	Tyr	Pro	Ile	Arg	Thr	Val	Ser	Gln	Leu	Thr	Arg	Glu
				165					170					175	

Ile Tyr Thr Asn Pro Val Leu Glu Asn Phe Asp Gly Ser Phe Arg Gly
180 185 190

Met Ala Gln Arg Ile Glu Gln Asn Ile Arg Gln Pro His Leu Met Asp
195 200 205

Ile Leu Asn Ser Ile Thr Ile Tyr Thr Asp Val His Arg Gly Phe Asn
210 215 220

Tyr Trp Ser Gly His Gln Ile Thr Ala Ser Pro Val Gly Phe Ser Gly
225 230 235 240

Pro Glu Phe

<210> 5
<211> 1959
<212> DNA
<213> Bacillus thuringiensis

<400> 5
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aatatcaatc cacttgttag cgcatcaaca gtccaaacgg gtataaacat agctggtaga 180
atattgggcg tattaggtgt gccgtttgct ggacaactag ctagttttta tagttttctt 240
gttggggaat tatggcctag tggtagagat ccatgggaaa ttttcctgga atatgtagaa 300
caacttataa gacaacaagt aacagaaaat actaggaata cggctattgc tcgattagaa 360
ggtctaggaa gaggctatag atcttaccag caggctcttg aaacttggtt agataaccga 420
aatgatgcaa gatcaagaag cattattctt gagcgctatg ttgctttaga acttgacatt 480
actactgcta taccgctttt cagaatacga aatgaagaag ttccattatt aatggtatat 540
gctcaagctg caaatttaca cctattatta ttgagagacg catccctttt tggtagtgaa 600
tgggggatgg catcttccga tgttaaccaa tattaccagg aacaaatcag atatacagag 660
gaatattcta accattgcgt acaatggtat aatacagggc taaataactt aagagggaca 720
aatgctgaaa gttggttgcg gtataatcaa ttccgtagag acctaacgtt aggggtatta 780
gatttagtag ccctattccc aagctatgat actcgcactt atccaatcaa tacgagtgct 840
cagttaacaa gagaaattta tacagatcca attgggagaa caaatgcacc ttcaggattt 900
gcaagtacga attggtttaa taataatgca ccatcgtttt ctgccataga ggctgccatt 960
ttcaggcctc cgcatctact tgattttcca gaacaactta caatttacag tgcatcaagc 1020
cgttggagta gcactcaaca tatgaattat tgggtgggac ataggcttaa cttccgccca 1080
ataggaggga cattaaatac ctcaacacaa ggacttacta ataatacttc aattaatcct 1140
gtaacattac attacgtttc gtctcgtgac gtttatagaa cagaatcaaa tgcagggaca 1200
aatatactat ttactactcc tgtgaatgga gtaccttggg ctagatttaa ttttataacc 1260
ctcagaatat ttatgaaaga ggcgccacta cctacagtca accgtatcag ggagttggga 1320
ttcaattatt tgattcagaa actgaattac caccagaaac aacagaacga ccaaattatg 1380
aatcatatag tcatagatat ctcatataga ctaatcatag gaaacacttt gagagcacca 1440
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ggatttactg gtggagacgt agttagattg aataggaata atggtaatat ccaaaataga 1620
gggtatattg aagttccaat tcaattcacg tcgacatcta ccagatatcg agttcgagta 1680
cgttatgctt ctgtaacctc gattgagctc aatgttaatt tgggcaattc atcaattttt 1740
acgaacacat taccagcaac agctgcatca ttagataatc tacaatcagg ggattttggt 1800
tatgttgaaa tcaacaatgc ttttacatcc gcaacaggta atatagtagg tgctagaaat 1860
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ttcgaggtag aatatgattt agaaagagca caaaaggcg 1959

<210> 6
<211> 653
<212> PRT
<213> Bacillus thuringiensis

<400> 6

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Glu Asn Glu Asn Glu Ile Ile Asn Ala Leu Ser Ile Pro Ala Val Ser
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Asn His Ser Ala Gln Met Asp Leu Ser Leu Asp Ala Arg Ile Glu Asp
            20                  25                  30

Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asn Pro Leu Val Ser Ala
        35                  40                  45

Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly Arg Ile Leu Gly Val
    50                  55                  60

Leu Gly Val Pro Phe Ala Gly Gln Leu Ala Ser Phe Tyr Ser Phe Leu
65                  70                  75                  80

Val Gly Glu Leu Trp Pro Ser Gly Arg Asp Pro Trp Glu Ile Phe Leu
                85                  90                  95

Glu Tyr Val Glu Gln Leu Ile Arg Gln Gln Val Thr Glu Asn Thr Arg
            100                 105                 110

Asn Thr Ala Ile Ala Arg Leu Glu Gly Leu Gly Arg Gly Tyr Arg Ser
        115                 120                 125

Tyr Gln Gln Ala Leu Glu Thr Trp Leu Asp Asn Arg Asn Asp Ala Arg
    130                 135                 140

Ser Arg Ser Ile Ile Leu Glu Arg Tyr Val Ala Leu Glu Leu Asp Ile
145                 150                 155                 160

Thr Thr Ala Ile Pro Leu Phe Arg Ile Arg Asn Glu Glu Val Pro Leu
                165                 170                 175

Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His Leu Leu Leu Leu Arg
            180                 185                 190

Asp Ala Ser Leu Phe Gly Ser Glu Trp Gly Met Ala Ser Ser Asp Val
        195                 200                 205

Asn Gln Tyr Tyr Gln Glu Gln Ile Arg Tyr Thr Glu Glu Tyr Ser Asn
    210                 215                 220

His Cys Val Gln Trp Tyr Asn Thr Gly Leu Asn Asn Leu Arg Gly Thr
225                 230                 235                 240

Asn Ala Glu Ser Trp Leu Arg Tyr Asn Gln Phe Arg Arg Asp Leu Thr
                245                 250                 255

Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro Ser Tyr Asp Thr Arg
            260                 265                 270
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Thr Tyr Pro Ile Asn Thr Ser Ala Gln Leu Thr Arg Glu Ile Tyr Thr
275 280 285

Asp Pro Ile Gly Arg Thr Asn Ala Pro Ser Gly Phe Ala Ser Thr Asn
290 295 300

Trp Phe Asn Asn Asn Ala Pro Ser Phe Ser Ala Ile Glu Ala Ala Ile
305 310 315 320

Phe Arg Pro Pro His Leu Leu Asp Phe Pro Glu Gln Leu Thr Ile Tyr
325 330 335

Ser Ala Ser Ser Arg Trp Ser Ser Thr Gln His Met Asn Tyr Trp Val
340 345 350

Gly His Arg Leu Asn Phe Arg Pro Ile Gly Gly Thr Leu Asn Thr Ser
355 360 365

Thr Gln Gly Leu Thr Asn Asn Thr Ser Ile Asn Pro Val Thr Leu His
370 375 380

Tyr Val Ser Ser Arg Asp Val Tyr Arg Thr Glu Ser Asn Ala Gly Thr
385 390 395 400

Asn Ile Leu Phe Thr Thr Pro Val Asn Gly Val Pro Trp Ala Arg Phe
405 410 415

Asn Phe Ile Thr Leu Arg Ile Phe Met Lys Glu Ala Pro Leu Pro Thr
420 425 430

Val Asn Arg Ile Arg Glu Leu Gly Phe Asn Tyr Leu Ile Gln Lys Leu
435 440 445

Asn Tyr His Gln Lys Gln Gln Asn Asp Gln Ile Met Asn His Ile Val
450 455 460

Ile Asp Ile Ser Tyr Arg Leu Ile Ile Gly Asn Thr Leu Arg Ala Pro
465 470 475 480

Val Tyr Ser Trp Thr His Arg Ser Ala Asp Arg Thr Asn Thr Ile Gly
485 490 495

Pro Asn Arg Ile Thr Gln Ile Pro Ala Val Lys Gly Arg Phe Leu Phe
500 505 510

Asn Gly Ser Val Ile Ser Gly Pro Gly Phe Thr Gly Gly Asp Val Val
515 520 525

Arg Leu Asn Arg Asn Asn Gly Asn Ile Gln Asn Arg Gly Tyr Ile Glu
530 535 540

Val Pro Ile Gln Phe Thr Ser Thr Ser Thr Arg Tyr Arg Val Arg Val
545 550 555 560

Arg Tyr Ala Ser Val Thr Ser Ile Glu Leu Asn Val Asn Leu Gly Asn
565 570 575

Ser Ser Ile Phe Thr Asn Thr Leu Pro Ala Thr Ala Ala Ser Leu Asp
580 585 590

Asn Leu Gln Ser Gly Asp Phe Gly Tyr Val Glu Ile Asn Asn Ala Phe
595 600 605

Thr Ser Ala Thr Gly Asn Ile Val Gly Ala Arg Asn Phe Ser Ala Asn
610 615 620

Ala Glu Val Ile Ile Asp Arg Phe Glu Phe Ile Pro Val Thr Ala Thr
625 630 635 640

Phe Glu Val Glu Tyr Asp Leu Glu Arg Ala Gln Lys Ala
645 650

<210> 7
<211> 328
<212> DNA
<213> Bacillus thuringiensis

<400> 7
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aggagtggtt cattaagtta ttcacagcaa acttcgtatg taatttcatt tccaaaaact 120
atggacgcag gtgaaccact aacatctcgt tcgttcgctt ttacaacaac cgtcactcca 180
atagccttta cacgagctca agaagaattt gatttataca tccaacagaa tgtttatata 240
gatagagttg aatttatccc agtagatgca acatttgagg caaaatctga tttagaaaga 300
gcgaaaaagg cggtgaatgc cttgttta 328

<210> 8
<211> 109
<212> PRT
<213> Bacillus thuringiensis

<400> 8
Leu Tyr Arg Lys Ile Thr Thr Lys Leu Leu Tyr Arg Phe Arg Tyr Ala
1 5 10 15

Ser Gly Ala Asn Arg Ser Gly Ser Leu Ser Tyr Ser Gln Gln Thr Ser
20 25 30

Tyr Val Ile Ser Phe Pro Lys Thr Met Asp Ala Gly Glu Pro Leu Thr
35 40 45

Ser Arg Ser Phe Ala Phe Thr Thr Thr Val Thr Pro Ile Ala Phe Thr
50 55 60

Arg Ala Gln Glu Glu Phe Asp Leu Tyr Ile Gln Gln Asn Val Tyr Ile
65 70 75 80

Asp Arg Val Glu Phe Ile Pro Val Asp Ala Thr Phe Glu Ala Lys Ser
85 90 95

Asp Leu Glu Arg Ala Lys Lys Ala Val Asn Ala Leu Phe
100 105

<210> 9
<211> 340
<212> DNA
<213> Bacillus thuringiensis

<400> 9
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tcgggagcaa ataggagtgg ttcattaagt tattcacagc aaacttcgta tgtaatttca 120
tttccaaaaa ctatggacgc aggtgaacca ctaacatctc gttcgttcgc ttttacaaca 180
accgtcactc caataacctt tacacgagct caagaagaat ttgatttata catccaacag 240
aatgtttata tagatagagt tgaatttatc ccagtagatg caacatttga ggcaaaatct 300
gatttagaaa gagcgaaaaa ggcggtgaat gccttgttta 340

<210> 10
<211> 113
<212> PRT
<213> Bacillus thuringiensis

<400> 10
Leu Arg Val Thr Phe Thr Gly Arg Leu Pro Gln Ser Tyr Tyr Ile Arg
1 5 10 15

Phe Arg Tyr Ala Ser Gly Ala Asn Arg Ser Gly Ser Leu Ser Tyr Ser
20 25 30

Gln Gln Thr Ser Tyr Val Ile Ser Phe Pro Lys Thr Met Asp Ala Gly
35 40 45

Glu Pro Leu Thr Ser Arg Ser Phe Ala Phe Thr Thr Thr Val Thr Pro
50 55 60

Ile Thr Phe Thr Arg Ala Gln Glu Glu Phe Asp Leu Tyr Ile Gln Gln
65 70 75 80

Asn Val Tyr Ile Asp Arg Val Glu Phe Ile Pro Val Asp Ala Thr Phe
85 90 95

Glu Ala Lys Ser Asp Leu Glu Arg Ala Lys Lys Ala Val Asn Ala Leu
100 105 110

Phe

<210> 11
<211> 306
<212> DNA
<213> Bacillus thuringiensis

<400> 11
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actactatta atattggtaa tttctcgagc actatggaca gtggggatga tttacagtac 120
ggaagattca gggttgcagg ttttactact ccatttacct tttcagatgc aaacagcaca 180
ttcacaatag gtgcttttgg cttctctcca aacaacgaag tttatataga tcgaattgaa 240
tttgtcccgg cagaagtaac atttgaggca gaatatgatt tagagaaagc tcagaaagcg 300

gtgaat 306

<210> 12
<211> 102
<212> PRT
<213> Bacillus thuringiensis

<400> 12
Val Ser Arg Glu Ile Val Cys Ser Thr Thr Asp Leu Gln Phe Tyr Thr
1 5 10 15

Asn Ile Asn Gly Thr Thr Ile Asn Ile Gly Asn Phe Ser Ser Thr Met
20 25 30

Asp Ser Gly Asp Asp Leu Gln Tyr Gly Arg Phe Arg Val Ala Gly Phe
35 40 45

Thr Thr Pro Phe Thr Phe Ser Asp Ala Asn Ser Thr Phe Thr Ile Gly
50 55 60

Ala Phe Gly Phe Ser Pro Asn Asn Glu Val Tyr Ile Asp Arg Ile Glu
65 70 75 80

Phe Val Pro Ala Glu Val Thr Phe Glu Ala Glu Tyr Asp Leu Glu Lys
85 90 95

Ala Gln Lys Ala Val Asn
100

<210> 13
<211> 279
<212> DNA
<213> Bacillus thuringiensis

<400> 13
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agtagtggga gtaatttaca gtccggaagc tttaggactg taggttttac tactccgttt 120
aacttttcaa atggatcaag tgtatttacg ttaagtgctc atgtcttcaa ttcaggcaat 180
gaagtttata tagatcgaat tgaatttatt ccggcagaag taacctttga ggcagaatat 240
gatttagaaa gagcacaaaa ggcggtgaat gagctgttt 279

<210> 14
<211> 93
<212> PRT
<213> Bacillus thuringiensis

<400> 14
Gln Phe His Thr Ser Ile Asp Gly Arg Pro Ile Asn Gln Gly Asn Phe
1 5 10 15

Ser Ala Thr Met Ser Ser Gly Ser Asn Leu Gln Ser Gly Ser Phe Arg
20 25 30

Thr Val Gly Phe Thr Thr Pro Phe Asn Phe Ser Asn Gly Ser Ser Val

35 40 45

Phe Thr Leu Ser Ala His Val Phe Asn Ser Gly Asn Glu Val Tyr Ile
50 55 60

Asp Arg Ile Glu Phe Ile Pro Ala Glu Val Thr Phe Glu Ala Glu Tyr
65 70 75 80

Asp Leu Glu Arg Ala Gln Lys Ala Val Asn Glu Leu Phe
85 90

<210> 15
<211> 397
<212> DNA
<213> Bacillus thuringiensis

<400> 15
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atgcgtgtaa acattactgc accactatca caaagatatc gcgtaagaat tcgctatgct 120
tctacgacag atttacaatt tttcacgaga atcaatggaa cttctgtaaa tcaaggtaat 180
ttccaaagaa ctatgaatag agggggtaat ttagaatctg gaaactttag gactgcagga 240
tttagtacgc cttttagttt tttcaaatgc gcaaagtaca ttcacattgg gtactcaggc 300
ttttcaaatc aggaagttta tatagatcga attgaatttg tcccggcaga agtaacattc 360
gaggcagaat ctgatttgga aagagcgcaa aaggcgg 397

<210> 16
<211> 132
<212> PRT
<213> Bacillus thuringiensis

<400> 16
Arg Thr Arg Phe Tyr Arg Trp Asp Ile Leu Arg Arg Thr Asn Val Gly
1 5 10 15

Ser Phe Gly Asp Met Arg Val Asn Ile Thr Ala Pro Leu Ser Gln Arg
20 25 30

Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr Asp Leu Gln Phe Phe
35 40 45

Thr Arg Ile Asn Gly Thr Ser Val Asn Gln Gly Asn Phe Gln Arg Thr
50 55 60

Met Asn Arg Gly Gly Asn Leu Glu Ser Gly Asn Phe Arg Thr Ala Gly
65 70 75 80

Phe Ser Thr Pro Phe Ser Phe Phe Lys Cys Ala Lys Tyr Ile His Ile
85 90 95

Gly Tyr Ser Gly Phe Ser Asn Gln Glu Val Tyr Ile Asp Arg Ile Glu
100 105 110

Phe Val Pro Ala Glu Val Thr Phe Glu Ala Glu Ser Asp Leu Glu Arg
115 120 125

Ala Gln Lys Ala
130

<210> 17
<211> 123
<212> DNA
<213> Bacillus thuringiensis

<400> 17
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acaggtaata tagtaggtgc tagaaatttt acgtgcaaat gcagaagtaa taatagacag 120
att 123

<210> 18
<211> 41
<212> PRT
<213> Bacillus thuringiensis

<400> 18
Ile Ile Tyr Asn Gln Gly Asp Phe Gly Tyr Val Glu Ile Asn Asn Ala
1 5 10 15

Phe Thr Ser Ala Thr Gly Asn Ile Val Gly Ala Arg Asn Phe Thr Cys
20 25 30

Lys Cys Arg Ser Asn Asn Arg Gln Ile
35 40

<210> 19
<211> 192
<212> DNA
<213> Bacillus thuringiensis

<400> 19
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tcacagccaa cttcatatgg aatttccttt ccaaaaacta tggatgcaga tgaatcatta 120
acatctcgtt catttgcact tgctacactt gctacaccgc taacctttag aaggcaagaa 180
gaattaaatc ta 192

<210> 20
<211> 64
<212> PRT
<213> Bacillus thuringiensis

<400> 20
Ser Tyr Tyr Ile Arg Phe Arg Tyr Ala Ser Val Ala Asn Arg Ser Gly
1 5 10 15

Ile Phe Ser Tyr Ser Gln Pro Thr Ser Tyr Gly Ile Ser Phe Pro Lys
20 25 30

Thr Met Asp Ala Asp Glu Ser Leu Thr Ser Arg Ser Phe Ala Leu Ala
35 40 45

Thr Leu Ala Thr Pro Leu Thr Phe Arg Arg Gln Glu Glu Leu Asn Leu
50 55 60

<210> 21
<211> 3507
<212> DNA
<213> Bacillus thuringiensis

<220>
<221> CDS
<222> (1)..(3507)

<400> 21
atg gag ata aat aat cag aac caa tgc ata cca tat aat tgc tta agt 48
Met Glu Ile Asn Asn Gln Asn Gln Cys Ile Pro Tyr Asn Cys Leu Ser
1 5 10 15

aat cct gag gaa gta ttt ttg gat ggg gag agg ata tta cct gat atc 96
Asn Pro Glu Glu Val Phe Leu Asp Gly Glu Arg Ile Leu Pro Asp Ile
20 25 30

gat cca ctc gaa gtt tct ttg tcg ctt ttg caa ttt ctt ttg aat aac 144
Asp Pro Leu Glu Val Ser Leu Ser Leu Leu Gln Phe Leu Leu Asn Asn
35 40 45

ttt gtt cca ggg ggg ggg ttt att tca gga tta ctt gat aaa ata tgg 192
Phe Val Pro Gly Gly Gly Phe Ile Ser Gly Leu Leu Asp Lys Ile Trp
50 55 60

ggg gct ttg aga cca tct gat tgg gaa tta ttt ctt gca cag att gaa 240
Gly Ala Leu Arg Pro Ser Asp Trp Glu Leu Phe Leu Ala Gln Ile Glu
65 70 75 80

cag ttg att gat cga aga ata gaa aga aca gta aga gca aaa gca atc 288
Gln Leu Ile Asp Arg Arg Ile Glu Arg Thr Val Arg Ala Lys Ala Ile
85 90 95

gct gaa tta gaa ggt tta ggg aga agt tat caa cta tat gga gag gca 336
Ala Glu Leu Glu Gly Leu Gly Arg Ser Tyr Gln Leu Tyr Gly Glu Ala
100 105 110

ttt aaa gag tgg gaa aaa act cca gat aac aca gcg gct cgg tct aga 384
Phe Lys Glu Trp Glu Lys Thr Pro Asp Asn Thr Ala Ala Arg Ser Arg
115 120 125

gta act gag aga ttt cgt ata att gat gct caa att gaa gca aat atc 432
Val Thr Glu Arg Phe Arg Ile Ile Asp Ala Gln Ile Glu Ala Asn Ile
130 135 140

cct tcg ttt cgg gtt tcc gga ttt gaa gtg cca ctt cta ttg gtt tat 480
Pro Ser Phe Arg Val Ser Gly Phe Glu Val Pro Leu Leu Leu Val Tyr
145 150 155 160

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acc caa gca gct aat ttg cat ctc gct cta tta aga gat tct gtt gtt   528
Thr Gln Ala Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ser Val Val
                165                 170                 175

ttt gga gag aga tgg gga ttg acg act aca aat gtc aat gat atc tat   576
Phe Gly Glu Arg Trp Gly Leu Thr Thr Thr Asn Val Asn Asp Ile Tyr
            180                 185                 190

aat aga caa gtt aat aga att ggt gaa tat agc aag cat tgt gta gat   624
Asn Arg Gln Val Asn Arg Ile Gly Glu Tyr Ser Lys His Cys Val Asp
        195                 200                 205

acg tat aaa aca gaa tta gaa cgt cta gga ttt aga tct ata gcg caa   672
Thr Tyr Lys Thr Glu Leu Glu Arg Leu Gly Phe Arg Ser Ile Ala Gln
    210                 215                 220

tgg aga ata tat aat cag ttt aga agg gaa ttg aca cta acg gta tta   720
Trp Arg Ile Tyr Asn Gln Phe Arg Arg Glu Leu Thr Leu Thr Val Leu
225                 230                 235                 240

gat att gtc gct gtt ttc ccg aac tat gat agt aga ctg tat ccg att   768
Asp Ile Val Ala Val Phe Pro Asn Tyr Asp Ser Arg Leu Tyr Pro Ile
                245                 250                 255

cga aca att tct caa ttg aca aga gaa att tat aca tcc cca gta agc   816
Arg Thr Ile Ser Gln Leu Thr Arg Glu Ile Tyr Thr Ser Pro Val Ser
            260                 265                 270

gaa ttt tat tat ggt gtc att aat agt aat aat ata att ggt acc ctt   864
Glu Phe Tyr Tyr Gly Val Ile Asn Ser Asn Asn Ile Ile Gly Thr Leu
        275                 280                 285

act gaa cag caa ata agg cga cca cat ctt atg gac ttc ttt aac tcc   912
Thr Glu Gln Gln Ile Arg Arg Pro His Leu Met Asp Phe Phe Asn Ser
    290                 295                 300

atg atc atg tat acg tca gat aat aga cga gaa cat tat tgg tca gga   960
Met Ile Met Tyr Thr Ser Asp Asn Arg Arg Glu His Tyr Trp Ser Gly
305                 310                 315                 320

ctt gaa atg acg gct act aat act gag gga cat caa agg tca ttc cct  1008
Leu Glu Met Thr Ala Thr Asn Thr Glu Gly His Gln Arg Ser Phe Pro
                325                 330                 335

tta gct ggg act ata ggg aat tca gct cca cca gta act gtt aga aat  1056
Leu Ala Gly Thr Ile Gly Asn Ser Ala Pro Pro Val Thr Val Arg Asn
            340                 345                 350

aat ggt gag gga att tat aga ata tta tcg gaa cca ttt tat tca gca  1104
Asn Gly Glu Gly Ile Tyr Arg Ile Leu Ser Glu Pro Phe Tyr Ser Ala
        355                 360                 365

cct ttt cta ggc aca agt gtg cta gga agt cgt ggg gaa gaa ttt gct  1152
Pro Phe Leu Gly Thr Ser Val Leu Gly Ser Arg Gly Glu Glu Phe Ala
    370                 375                 380
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ttt gca tct aat act act aca agt ctg cca tct aca ata tat aga aat   1200
Phe Ala Ser Asn Thr Thr Thr Ser Leu Pro Ser Thr Ile Tyr Arg Asn
385                 390                 395                 400

cgt gga aca gta gat tca tta gtc agc ata ccg cca cag gat tat agc   1248
Arg Gly Thr Val Asp Ser Leu Val Ser Ile Pro Pro Gln Asp Tyr Ser
                405                 410                 415

gta cca ccg cac agg ggg tat agt cat tta tta agt cac gtt acg atg   1296
Val Pro Pro His Arg Gly Tyr Ser His Leu Leu Ser His Val Thr Met
            420                 425                 430

cgc aat agt tct cct ata ttc cac tgg aca cat cgt agt gca acc cct   1344
Arg Asn Ser Ser Pro Ile Phe His Trp Thr His Arg Ser Ala Thr Pro
        435                 440                 445

aga aat aca att gat cca gat agt atc act caa att cca gca gtt aag   1392
Arg Asn Thr Ile Asp Pro Asp Ser Ile Thr Gln Ile Pro Ala Val Lys
    450                 455                 460

gga gcg tat att ttt aat agt cca gtc att act ggg cca gga cat aca   1440
Gly Ala Tyr Ile Phe Asn Ser Pro Val Ile Thr Gly Pro Gly His Thr
465                 470                 475                 480

ggt ggg gat ata ata agg ttt aac cct aat act cag aac aac ata aga   1488
Gly Gly Asp Ile Ile Arg Phe Asn Pro Asn Thr Gln Asn Asn Ile Arg
                485                 490                 495

att cca ttt caa tca aat gcg gta cag cgt tat cga att aga atg cgt   1536
Ile Pro Phe Gln Ser Asn Ala Val Gln Arg Tyr Arg Ile Arg Met Arg
            500                 505                 510

tat gcg gca gaa gct gat tgt att tta gaa agt gga gta aac att gtt   1584
Tyr Ala Ala Glu Ala Asp Cys Ile Leu Glu Ser Gly Val Asn Ile Val
        515                 520                 525

act ggg gca ggg gtc acc ttt agg cca att cct att aaa gct aca atg   1632
Thr Gly Ala Gly Val Thr Phe Arg Pro Ile Pro Ile Lys Ala Thr Met
    530                 535                 540

act cct gga agt cct tta aca tat tac agc ttc cag tat gca gat tta   1680
Thr Pro Gly Ser Pro Leu Thr Tyr Tyr Ser Phe Gln Tyr Ala Asp Leu
545                 550                 555                 560

aat ata aat ctt act gcg ccg ata aga cct aat aat ttt gta tct att   1728
Asn Ile Asn Leu Thr Ala Pro Ile Arg Pro Asn Asn Phe Val Ser Ile
                565                 570                 575

aga cgt tca aac caa cca gga aac ctt tat ata gat aga att gaa ttc   1776
Arg Arg Ser Asn Gln Pro Gly Asn Leu Tyr Ile Asp Arg Ile Glu Phe
            580                 585                 590

att cca att gac cca atc cgt gag gca gaa cat gat tta gaa aga gcg   1824
Ile Pro Ile Asp Pro Ile Arg Glu Ala Glu His Asp Leu Glu Arg Ala
        595                 600                 605

caa aag gcg gtg aat gcg ctg ttt act tct tcc aat caa cta gga tta   1872
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Gln Lys Ala Val Asn Ala Leu Phe Thr Ser Ser Asn Gln Leu Gly Leu
    610                 615                 620

aaa aca gat gtg acg gat tat cat att gat caa gtg tcc aat tta gtt   1920
Lys Thr Asp Val Thr Asp Tyr His Ile Asp Gln Val Ser Asn Leu Val
625                 630                 635                 640

gcg tgt tta tcg gat gaa ttc tgc ctg gat gaa aag cga gaa ttg tcc   1968
Ala Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu Lys Arg Glu Leu Ser
                645                 650                 655

gag aaa gtt aaa cat gcg aag cga ctc agt gat gag aga aat tta ctc   2016
Glu Lys Val Lys His Ala Lys Arg Leu Ser Asp Glu Arg Asn Leu Leu
            660                 665                 670

caa gat caa aac ttt aca ggc atc aat agg caa gta gac cgt ggg tgg   2064
Gln Asp Gln Asn Phe Thr Gly Ile Asn Arg Gln Val Asp Arg Gly Trp
        675                 680                 685

aga gga agt acg gat att acc atc caa gga ggg aat gat gta ttc aaa   2112
Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asn Asp Val Phe Lys
    690                 695                 700

gag aat tac gtc aca cta cca ggt acc ttt gat gag tgt tac cca acg   2160
Glu Asn Tyr Val Thr Leu Pro Gly Thr Phe Asp Glu Cys Tyr Pro Thr
705                 710                 715                 720

tat ttg tat caa aaa ata gat gag tca aaa tta aaa cct tat act cgc   2208
Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys Pro Tyr Thr Arg
                725                 730                 735

tat gaa tta aga ggg tat att gaa gat agt caa gac tta gaa gtc tat   2256
Tyr Glu Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu Val Tyr
            740                 745                 750

ttg atc cgt tac aat gca aaa cac gaa acg tta aat gtg cca ggt acg   2304
Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Leu Asn Val Pro Gly Thr
        755                 760                 765

ggt tcc tta tgg cca ctt gca gcc gaa agt tca atc ggg agg tgc ggc   2352
Gly Ser Leu Trp Pro Leu Ala Ala Glu Ser Ser Ile Gly Arg Cys Gly
    770                 775                 780

gaa ccg aat cga tgc gcg cca cat att gaa tgg aat cct gaa cta gat   2400
Glu Pro Asn Arg Cys Ala Pro His Ile Glu Trp Asn Pro Glu Leu Asp
785                 790                 795                 800

tgt tcg tgt agg gat gga gaa aaa tgt gca cat cat tct cat cat ttc   2448
Cys Ser Cys Arg Asp Gly Glu Lys Cys Ala His His Ser His His Phe
                805                 810                 815

tcc ttg gat att gat gtt gga tgt aca gac tta aat gag gat tta ggt   2496
Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Asn Glu Asp Leu Gly
            820                 825                 830

gta tgg gtg ata ttt aag att aag acg caa gat ggc tat gca aga cta   2544
Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp Gly Tyr Ala Arg Leu
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          835                 840                 845

gga aat tta gag ttt ctc gaa gag aaa cca ttg tta gga gaa gcg cta   2592
Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu Leu Gly Glu Ala Leu
     850                 855                 860

gct cgt gtg aag aga gcg gag aaa aaa tgg aga gac aaa cgc gac aaa   2640
Ala Arg Val Lys Arg Ala Glu Lys Lys Trp Arg Asp Lys Arg Asp Lys
865                 870                 875                 880

ttg gaa tgg gaa aca aat att gtt tat aaa gag gca aaa gaa tct gta   2688
Leu Glu Trp Glu Thr Asn Ile Val Tyr Lys Glu Ala Lys Glu Ser Val
                885                 890                 895

gat gct tta ttc gta gat tct caa tat aat aga tta caa acg gat acg   2736
Asp Ala Leu Phe Val Asp Ser Gln Tyr Asn Arg Leu Gln Thr Asp Thr
            900                 905                 910

aac att gcg atg att cat gtg gca gat aaa cgc gtt cat cga atc cga   2784
Asn Ile Ala Met Ile His Val Ala Asp Lys Arg Val His Arg Ile Arg
        915                 920                 925

gaa gcg tat ttg cca gag tta tct gtg att ccg ggt gtc aat gcg gct   2832
Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly Val Asn Ala Ala
    930                 935                 940

att ttc gaa gaa tta gaa ggt ctt att ttc act gca ttc tcc cta tat   2880
Ile Phe Glu Glu Leu Glu Gly Leu Ile Phe Thr Ala Phe Ser Leu Tyr
945                 950                 955                 960

gat gcg aga aat gtc att aaa aac gga gat ttc aat cat ggt tta tca   2928
Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn His Gly Leu Ser
                965                 970                 975

tgc tgg aac gtg aaa ggg cat gta gat gta gaa gaa caa aat aac cac   2976
Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu Gln Asn Asn His
            980                 985                 990

cgt tcg gtc ctt gtt gtt ccg gaa tgg gaa gca gaa gtg tca caa gaa   3024
Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu Val Ser Gln Glu
        995                1000                1005

gtc cgc gta tgt cca gga cgt ggc tat atc ctg cgt gtt aca gcg tac   3072
Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr Ala Tyr
   1010                1015                1020

aaa gag ggc tac gga gaa gga tgc gta acg atc cat gaa att gaa gat   3120
Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile His Glu Ile Glu Asp
1025                1030                1035                1040

cat aca gac gaa ctg aaa ttt aga aac tgt gaa gaa gag gaa gtg tat   3168
His Thr Asp Glu Leu Lys Phe Arg Asn Cys Glu Glu Glu Glu Val Tyr
                1045                1050                1055

ccg aat aac acg gta acg tgt aat gat tat cca gca aat caa gaa gaa   3216
Pro Asn Asn Thr Val Thr Cys Asn Asp Tyr Pro Ala Asn Gln Glu Glu
            1060                1065                1070
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tac agg gct gcg gaa act tcc cgt aat cgt gga tat ggc gaa tct tat   3264
Tyr Arg Ala Ala Glu Thr Ser Arg Asn Arg Gly Tyr Gly Glu Ser Tyr
      1075                1080                1085

gaa agt aat tct tcc ata cca gct gag tat gcg cca att tat gag aaa   3312
Glu Ser Asn Ser Ser Ile Pro Ala Glu Tyr Ala Pro Ile Tyr Glu Lys
   1090                1095                1100

gca tat aca gat gga aga aaa gag aat tct tgt gaa tct aac aga gga   3360
Ala Tyr Thr Asp Gly Arg Lys Glu Asn Ser Cys Glu Ser Asn Arg Gly
1105                1110                1115                1120

tat gga aat tac aca ccg tta cca gca ggt tat gtg aca aaa gaa tta   3408
Tyr Gly Asn Tyr Thr Pro Leu Pro Ala Gly Tyr Val Thr Lys Glu Leu
                1125                1130                1135

gag tac ttc cca gaa acc gat aag gta tgg ata gag att gga gaa acg   3456
Glu Tyr Phe Pro Glu Thr Asp Lys Val Trp Ile Glu Ile Gly Glu Thr
            1140                1145                1150

gaa gga aca ttc atc gta gac agt gtg gaa tta ctc ctc atg gag gaa   3504
Glu Gly Thr Phe Ile Val Asp Ser Val Glu Leu Leu Leu Met Glu Glu
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tag                                                              3507
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<213> Bacillus thuringiensis

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Asn Pro Glu Glu Val Phe Leu Asp Gly Glu Arg Ile Leu Pro Asp Ile
            20                  25                  30
Asp Pro Leu Glu Val Ser Leu Ser Leu Leu Gln Phe Leu Leu Asn Asn
        35                  40                  45
Phe Val Pro Gly Gly Gly Phe Ile Ser Gly Leu Leu Asp Lys Ile Trp
    50                  55                  60
Gly Ala Leu Arg Pro Ser Asp Trp Glu Leu Phe Leu Ala Gln Ile Glu
65                  70                  75                  80
Gln Leu Ile Asp Arg Arg Ile Glu Arg Thr Val Arg Ala Lys Ala Ile
                85                  90                  95
Ala Glu Leu Glu Gly Leu Gly Arg Ser Tyr Gln Leu Tyr Gly Glu Ala
            100                 105                 110
Phe Lys Glu Trp Glu Lys Thr Pro Asp Asn Thr Ala Ala Arg Ser Arg
        115                 120                 125
Val Thr Glu Arg Phe Arg Ile Ile Asp Ala Gln Ile Glu Ala Asn Ile
    130                 135                 140
Pro Ser Phe Arg Val Ser Gly Phe Glu Val Pro Leu Leu Leu Val Tyr
145                 150                 155                 160
Thr Gln Ala Ala Asn Leu His Leu Ala Leu Leu Arg Asp Ser Val Val
                165                 170                 175
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Phe Gly Glu Arg Trp Gly Leu Thr Thr Thr Asn Val Asn Asp Ile Tyr
180 185 190
Asn Arg Gln Val Asn Arg Ile Gly Glu Tyr Ser Lys His Cys Val Asp
195 200 205
Thr Tyr Lys Thr Glu Leu Glu Arg Leu Gly Phe Arg Ser Ile Ala Gln
210 215 220
Trp Arg Ile Tyr Asn Gln Phe Arg Arg Glu Leu Thr Leu Thr Val Leu
225 230 235 240
Asp Ile Val Ala Val Phe Pro Asn Tyr Asp Ser Arg Leu Tyr Pro Ile
245 250 255
Arg Thr Ile Ser Gln Leu Thr Arg Glu Ile Tyr Thr Ser Pro Val Ser
260 265 270
Glu Phe Tyr Tyr Gly Val Ile Asn Ser Asn Asn Ile Ile Gly Thr Leu
275 280 285
Thr Glu Gln Gln Ile Arg Arg Pro His Leu Met Asp Phe Phe Asn Ser
290 295 300
Met Ile Met Tyr Thr Ser Asp Asn Arg Arg Glu His Tyr Trp Ser Gly
305 310 315 320
Leu Glu Met Thr Ala Thr Asn Thr Glu Gly His Gln Arg Ser Phe Pro
325 330 335
Leu Ala Gly Thr Ile Gly Asn Ser Ala Pro Pro Val Thr Val Arg Asn
340 345 350
Asn Gly Glu Gly Ile Tyr Arg Ile Leu Ser Glu Pro Phe Tyr Ser Ala
355 360 365
Pro Phe Leu Gly Thr Ser Val Leu Gly Ser Arg Gly Glu Glu Phe Ala
370 375 380
Phe Ala Ser Asn Thr Thr Thr Ser Leu Pro Ser Thr Ile Tyr Arg Asn
385 390 395 400
Arg Gly Thr Val Asp Ser Leu Val Ser Ile Pro Pro Gln Asp Tyr Ser
405 410 415
Val Pro Pro His Arg Gly Tyr Ser His Leu Leu Ser His Val Thr Met
420 425 430
Arg Asn Ser Ser Pro Ile Phe His Trp Thr His Arg Ser Ala Thr Pro
435 440 445
Arg Asn Thr Ile Asp Pro Asp Ser Ile Thr Gln Ile Pro Ala Val Lys
450 455 460
Gly Ala Tyr Ile Phe Asn Ser Pro Val Ile Thr Gly Pro Gly His Thr
465 470 475 480
Gly Gly Asp Ile Ile Arg Phe Asn Pro Asn Thr Gln Asn Asn Ile Arg
485 490 495
Ile Pro Phe Gln Ser Asn Ala Val Gln Arg Tyr Arg Ile Arg Met Arg
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Tyr Ala Ala Glu Ala Asp Cys Ile Leu Glu Ser Gly Val Asn Ile Val
515 520 525
Thr Gly Ala Gly Val Thr Phe Arg Pro Ile Pro Ile Lys Ala Thr Met
530 535 540
Thr Pro Gly Ser Pro Leu Thr Tyr Tyr Ser Phe Gln Tyr Ala Asp Leu
545 550 555 560
Asn Ile Asn Leu Thr Ala Pro Ile Arg Pro Asn Asn Phe Val Ser Ile
565 570 575
Arg Arg Ser Asn Gln Pro Gly Asn Leu Tyr Ile Asp Arg Ile Glu Phe
580 585 590
Ile Pro Ile Asp Pro Ile Arg Glu Ala Glu His Asp Leu Glu Arg Ala
595 600 605
Gln Lys Ala Val Asn Ala Leu Phe Thr Ser Ser Asn Gln Leu Gly Leu
610 615 620
Lys Thr Asp Val Thr Asp Tyr His Ile Asp Gln Val Ser Asn Leu Val

625 630 635 640
Ala Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu Lys Arg Glu Leu Ser
645 650 655
Glu Lys Val Lys His Ala Lys Arg Leu Ser Asp Glu Arg Asn Leu Leu
660 665 670
Gln Asp Gln Asn Phe Thr Gly Ile Asn Arg Gln Val Asp Arg Gly Trp
675 680 685
Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asn Asp Val Phe Lys
690 695 700
Glu Asn Tyr Val Thr Leu Pro Gly Thr Phe Asp Glu Cys Tyr Pro Thr
705 710 715 720
Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys Pro Tyr Thr Arg
725 730 735
Tyr Glu Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu Val Tyr
740 745 750
Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Leu Asn Val Pro Gly Thr
755 760 765
Gly Ser Leu Trp Pro Leu Ala Ala Glu Ser Ser Ile Gly Arg Cys Gly
770 775 780
Glu Pro Asn Arg Cys Ala Pro His Ile Glu Trp Asn Pro Glu Leu Asp
785 790 795 800
Cys Ser Cys Arg Asp Gly Glu Lys Cys Ala His His Ser His His Phe
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850 855 860
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865 870 875 880
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885 890 895
Asp Ala Leu Phe Val Asp Ser Gln Tyr Asn Arg Leu Gln Thr Asp Thr
900 905 910
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915 920 925
Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly Val Asn Ala Ala
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945 950 955 960
Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn His Gly Leu Ser
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Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu Val Ser Gln Glu
995 1000 1005
Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr Ala Tyr
1010 1015 1020
Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile His Glu Ile Glu Asp
1025 1030 1035 1040
His Thr Asp Glu Leu Lys Phe Arg Asn Cys Glu Glu Glu Glu Val Tyr
1045 1050 1055
Pro Asn Asn Thr Val Thr Cys Asn Asp Tyr Pro Ala Asn Gln Glu Glu
1060 1065 1070
Tyr Arg Ala Ala Glu Thr Ser Arg Asn Arg Gly Tyr Gly Glu Ser Tyr
1075 1080 1085

Glu Ser Asn Ser Ser Ile Pro Ala Glu Tyr Ala Pro Ile Tyr Glu Lys
1090 1095 1100
Ala Tyr Thr Asp Gly Arg Lys Glu Asn Ser Cys Glu Ser Asn Arg Gly
1105 1110 1115 1120
Tyr Gly Asn Tyr Thr Pro Leu Pro Ala Gly Tyr Val Thr Lys Glu Leu
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Thr Arg Tyr Arg Val Arg Val Arg Tyr Ala Ser Ala Thr Pro Ile Gln
20 25 30

Val Asn Val His Trp Glu Asn Ser Ser Phe Phe Ser Gly Thr Val Pro
35 40 45

Ala Thr Ala Gln Ser Leu Asp Asn Leu Gln Ser Asn Asn Phe Gly Tyr
50 55 60

Phe Glu Thr Ala Asn Thr Ile Ser Ser Ser Leu Asp Gly Ile Val Gly
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Ile Arg Asn Phe Ser Ala Asn Ala Asp Leu Ile Ile Asp Arg Phe Glu
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attccagtta ctgcaacatt tgaggcagaa tatgatttag aaagagcgca aagggcggtg 180
aatgcc 186

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<213> Bacillus thuringiensis

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Ala Glu Tyr Asp Leu Glu Arg Ala Gln Arg Ala Val Asn Ala
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gtaaaaaatg taccagataa tttagtatct gatacttact cagatggttc ttgcagcgga 2520
atcaaccgtt gtgatgaaca gcatcaggta gatatgcagc tagatgcgga gcatcatcca 2580
atggattgct gtgaagcggc tcaaacacat gagttttctt cctatattaa tacaggggat 2640
ctaaatgcaa gtgtagatca gggcatttgg gttgtattaa aagttcgaac aacagatggg 2700
tatgcgacgt taggaaatct tgaattggta gaggttgggc cattatcggg tgaatctcta 2760
gaacgggaac aaagagataa tgcgaaatgg aatgcagagc taggaagaaa acgtgcagaa 2820
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gatcaacaat taaatccaga aattgggcta gcagaaatta atgaagcttc aaatcttgta 2940
gagtcaattt cgggtgtata tagtgataca ctattacaga ttcctgggat taactacgaa 3000
atttacacag agttatccga tcgcttacaa caagcatcgt atctgtatac gtctagaaat 3060
gcggtgcaaa atggagactt taacagtggt ctagatagtt ggaatacaac tatggatgca 3120
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acatttaatg catgtgacta cgatgtaaat ggtacgtatg tcaatgacaa ttcgtatata 3360
acagaagaag tggtattcta cccagagaca aaacatatgt gggtagaggt gagtgaatcc 3420
gaaggttcat tctatataga cagtattgag tttattgaaa cacaagagta g 3471

<210> 28
<211> 1156
<212> PRT
<213> Bacillus thuringiensis

<400> 28
Met Asn Arg Asn Asn Gln Asn Glu Tyr Glu Ile Ile Asp Ala Pro His
1 5 10 15

Cys Gly Cys Pro Ser Asp Asp Asp Val Arg Tyr Pro Leu Ala Ser Asp
20 25 30

Pro Asn Ala Ala Leu Gln Asn Met Asn Tyr Lys Asp Tyr Leu Gln Met
35 40 45

Thr Asp Glu Asp Tyr Thr Asp Ser Tyr Ile Asn Pro Ser Leu Ser Ile

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     50                  55                  60

Ser Gly Arg Asp Ala Val Gln Thr Ala Leu Thr Val Val Gly Arg Ile
 65                  70                  75                  80

Leu Gly Ala Leu Gly Val Pro Phe Ser Gly Gln Ile Val Ser Phe Tyr
                 85                  90                  95

Gln Phe Leu Leu Asn Thr Leu Trp Pro Val Asn Asp Thr Ala Ile Trp
            100                 105                 110

Glu Ala Phe Met Arg Gln Val Glu Glu Leu Val Asn Gln Gln Ile Thr
        115                 120                 125

Glu Phe Ala Arg Asn Gln Ala Leu Ala Arg Leu Gln Gly Leu Gly Asp
    130                 135                 140

Ser Phe Asn Val Tyr Gln Arg Ser Leu Gln Asn Trp Leu Ala Asp Arg
145                 150                 155                 160

Asn Asp Thr Arg Asn Leu Ser Val Val Arg Ala Gln Phe Ile Ala Leu
                165                 170                 175

Asp Leu Asp Phe Val Asn Ala Ile Pro Leu Phe Ala Val Asn Gly Gln
            180                 185                 190

Gln Val Pro Leu Leu Ser Val Tyr Ala Gln Ala Val Asn Leu His Leu
        195                 200                 205

Leu Leu Leu Lys Asp Ala Ser Leu Phe Gly Glu Gly Trp Gly Phe Thr
    210                 215                 220

Gln Gly Glu Ile Ser Thr Tyr Tyr Asp Arg Gln Leu Glu Leu Thr Ala
225                 230                 235                 240

Lys Tyr Thr Asn Tyr Cys Glu Thr Trp Tyr Asn Thr Gly Leu Asp Arg
                245                 250                 255

Leu Arg Gly Thr Asn Thr Glu Ser Trp Leu Arg Tyr His Gln Phe Arg
            260                 265                 270

Arg Glu Met Thr Leu Val Val Leu Asp Val Val Ala Leu Phe Pro Tyr
        275                 280                 285

Tyr Asp Val Arg Leu Tyr Pro Thr Gly Ser Asn Pro Gln Leu Thr Arg
    290                 295                 300

Glu Val Tyr Thr Asp Pro Ile Val Phe Asn Pro Pro Ala Asn Val Gly
305                 310                 315                 320

Leu Cys Arg Arg Trp Gly Thr Asn Pro Tyr Asn Thr Phe Ser Glu Leu
                325                 330                 335

Glu Asn Ala Phe Ile Arg Pro Pro His Leu Phe Asp Arg Leu Asn Ser
            340                 345                 350

Leu Thr Ile Ser Ser Asn Arg Phe Pro Val Ser Ser Asn Phe Met Asp
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          355                 360                 365

Tyr Trp Ser Gly His Thr Leu Arg Arg Ser Tyr Leu Asn Asp Ser Ala
    370                 375                 380

Val Gln Glu Asp Ser Tyr Gly Leu Ile Thr Thr Thr Arg Ala Thr Ile
385                 390                 395                 400

Asn Pro Gly Val Asp Gly Thr Asn Arg Ile Glu Ser Thr Ala Val Asp
                405                 410                 415

Phe Arg Ser Ala Leu Ile Gly Ile Tyr Gly Val Asn Arg Ala Ser Phe
            420                 425                 430

Val Pro Gly Gly Leu Phe Asn Gly Thr Thr Ser Pro Ala Asn Gly Gly
        435                 440                 445

Cys Arg Asp Leu Tyr Asp Thr Asn Asp Glu Leu Pro Pro Asp Glu Ser
    450                 455                 460

Thr Gly Ser Ser Thr His Arg Leu Ser His Val Thr Phe Phe Ser Phe
465                 470                 475                 480

Gln Thr Asn Gln Ala Gly Ser Ile Ala Asn Ala Gly Ser Val Pro Thr
                485                 490                 495

Tyr Val Trp Thr Arg Arg Asp Val Asp Leu Asn Asn Thr Ile Thr Pro
            500                 505                 510

Asn Arg Ile Thr Gln Leu Pro Leu Val Lys Ala Ser Ala Pro Val Ser
        515                 520                 525

Gly Thr Thr Val Leu Lys Gly Pro Gly Phe Thr Gly Gly Gly Ile Leu
    530                 535                 540

Arg Arg Thr Thr Asn Gly Thr Phe Gly Thr Leu Arg Val Thr Val Asn
545                 550                 555                 560

Ser Pro Leu Thr Gln Arg Tyr Arg Val Arg Val Arg Phe Ala Ser Ser
                565                 570                 575

Gly Asn Phe Ser Ile Arg Ile Leu Arg Gly Asn Thr Ser Ile Ala Tyr
            580                 585                 590

Gln Arg Phe Gly Ser Thr Met Asn Arg Gly Gln Glu Leu Thr Tyr Glu
        595                 600                 605

Ser Phe Val Thr Ser Glu Phe Thr Thr Asn Gln Ser Asp Leu Pro Phe
    610                 615                 620

Thr Phe Thr Gln Ala Gln Glu Asn Leu Thr Ile Leu Ala Glu Gly Val
625                 630                 635                 640

Ser Thr Gly Ser Glu Tyr Phe Ile Asp Arg Ile Glu Ile Ile Pro Val
                645                 650                 655

Asn Pro Ala Arg Glu Ala Glu Glu Asp Leu Glu Ala Ala Lys Lys Ala
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660 665 670

Val Ala Asn Leu Phe Thr Arg Thr Arg Asp Gly Leu Gln Val Asn Val
675 680 685

Thr Asp Tyr Gln Val Asp Gln Ala Ala Asn Leu Val Ser Cys Leu Ser
690 695 700

Asp Glu Gln Tyr Gly His Asp Lys Lys Met Leu Leu Glu Ala Val Arg
705 710 715 720

Ala Ala Lys Arg Leu Ser Arg Glu Arg Asn Leu Leu Gln Asp Pro Asp
725 730 735

Phe Asn Thr Ile Asn Ser Thr Glu Glu Asn Gly Trp Lys Ala Ser Asn
740 745 750

Gly Val Thr Ile Ser Glu Gly Gly Pro Phe Phe Lys Gly Arg Ala Leu
755 760 765

Gln Leu Ala Ser Ala Arg Glu Asn Tyr Pro Thr Tyr Ile Tyr Gln Lys
770 775 780

Val Asp Ala Ser Val Leu Lys Pro Tyr Thr Arg Tyr Arg Leu Asp Gly
785 790 795 800

Phe Val Lys Ser Ser Gln Asp Leu Glu Ile Asp Leu Ile His His His
805 810 815

Lys Val His Leu Val Lys Asn Val Pro Asp Asn Leu Val Ser Asp Thr
820 825 830

Tyr Ser Asp Gly Ser Cys Ser Gly Ile Asn Arg Cys Asp Glu Gln His
835 840 845

Gln Val Asp Met Gln Leu Asp Ala Glu His His Pro Met Asp Cys Cys
850 855 860

Glu Ala Ala Gln Thr His Glu Phe Ser Ser Tyr Ile Asn Thr Gly Asp
865 870 875 880

Leu Asn Ala Ser Val Asp Gln Gly Ile Trp Val Val Leu Lys Val Arg
885 890 895

Thr Thr Asp Gly Tyr Ala Thr Leu Gly Asn Leu Glu Leu Val Glu Val
900 905 910

Gly Pro Leu Ser Gly Glu Ser Leu Glu Arg Glu Gln Arg Asp Asn Ala
915 920 925

Lys Trp Asn Ala Glu Leu Gly Arg Lys Arg Ala Glu Ile Asp Arg Val
930 935 940

Tyr Leu Ala Ala Lys Gln Ala Ile Asn His Leu Phe Val Asp Tyr Gln
945 950 955 960

Asp Gln Gln Leu Asn Pro Glu Ile Gly Leu Ala Glu Ile Asn Glu Ala

965 970 975

Ser Asn Leu Val Glu Ser Ile Ser Gly Val Tyr Ser Asp Thr Leu Leu
980 985 990

Gln Ile Pro Gly Ile Asn Tyr Glu Ile Tyr Thr Glu Leu Ser Asp Arg
995 1000 1005

Leu Gln Gln Ala Ser Tyr Leu Tyr Thr Ser Arg Asn Ala Val Gln Asn
1010 1015 1020

Gly Asp Phe Asn Ser Gly Leu Asp Ser Trp Asn Thr Thr Met Asp Ala
1025 1030 1035 1040

Ser Val Gln Gln Asp Gly Asn Met His Phe Leu Val Leu Ser His Trp
1045 1050 1055

Asp Ala Gln Val Ser Gln Gln Leu Arg Val Asn Pro Asn Cys Lys Tyr
1060 1065 1070

Val Leu Arg Val Thr Ala Arg Lys Val Gly Gly Gly Asp Gly Tyr Val
1075 1080 1085

Thr Ile Arg Asp Gly Ala His His Gln Glu Thr Leu Thr Phe Asn Ala
1090 1095 1100

Cys Asp Tyr Asp Val Asn Gly Thr Tyr Val Asn Asp Asn Ser Tyr Ile
1105 1110 1115 1120

Thr Glu Glu Val Val Phe Tyr Pro Glu Thr Lys His Met Trp Val Glu
1125 1130 1135

Val Ser Glu Ser Glu Gly Ser Phe Tyr Ile Asp Ser Ile Glu Phe Ile
1140 1145 1150

Glu Thr Gln Glu
1155

<210> 29
<211> 2407
<212> DNA
<213> Bacillus thuringiensis

<400> 29
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gttgcgaaat atcctttagc caacaatcca tattcatctg ctttaaattt aaattcttgt 120
caaaatagta gtattctcaa ctggattaac ataataggcg atgcagcaaa agaagcagta 180
tctattggga caaccatagt ctctcttatc acagcacctt ctcttactgg attaatttca 240
atagtatatg accttatagg taaagtacta ggaggtagta gtggacaatc catatcagat 300
ttgtctatat gtgacttatt atctattatt gatttacggg taagtcagag tgttttaaat 360
gatgggattg cagattttaa tggttctgta ctcttataca ggaactattt agaggctctg 420
gatagctgga ataagaatcc taattctgct tctgctgaag aactccgtac tcgttttaga 480
atcgccgact cagaatttga tagaatttta acccgagggt ctttaacgaa tggtggctcg 540
ttagctagac aaaatgccca aatattatta ttaccttctt ttgcgagcgc tgcatttttc 600
catttattac tactaaggga tgctactaga tatggcacta attgggggct atacaatgct 660
acacctttta taaattatca atcaaaacta gtagagctta ttgaactata tactgattat 720

tgcgtacatt gggataatcg aggttcaacc gaactaagac aacgagggcc tagtgctaca 780
gcttggttag aatttcatag atatcggaga gagatgacat tgatgggatt agaaatagta 840
gcatcatttt caagtcttga tattactaat tacccaatag aaacagattt tcagttgagt 900
agggtcattt atacagatcc aattggtttt gtacatcgta gtagtcttag gggagaaagt 960
tggtttagct ttgttaatag agctaatttc tcagatttag aaaatgcaat acctaatcct 1020
agaccgtctt ggtttttaaa taatatgatt atatctactg gttcacttac attgccggtt 1080
agcccaagta ctgatagagc gagggtatgg tatggaagtc gagatcgaat ttcccctgct 1140
aattcacaat ttattactga actaatctct ggacaacata cgactgctac acaaactatt 1200
ttagggcgaa atatatttag agtagattct caagcttgta atttaaatga taccacatat 1260
ggagtgaata gggcggtatt ttatcatgat gcgagtgaag gttctcaaag atccgtgtac 1320
gaggggtata ttcgaacaac tgggatagat aaccctagag ttcaaaatat taacacttat 1380
ttacctggag aaaattcaga tatcccaact ccagaagact atactcatat attaagcaca 1440
acaataaatt taacaggagg acttagacaa gtagcatcta atcgccgttc atctttagta 1500
atgtatggtt ggacacataa aagtctggct cgtaacaata ccattaatcc agatagaatt 1560
acacagatac cattgacgaa ggttgatacc cgaggcacag gtgtttctta tgtgaatgat 1620
ccaggattta taggaggagc tctacttcaa aggactgacc atggttcgct tggagtattg 1680
agggtccaat ttccacttca cttaagacaa caatatcgta ttagagtccg ttatgcttct 1740
acaacaaata ttcgattgag tgtgaatggc agtttcggta ctatttctca aaatctccct 1800
agtacaatga gattaggaga ggatttaaga tacggatctt ttgctataag agagtttaat 1860
acttctatta gacccactgc aagtccggac caaattcgat tgacaataga accatctttt 1920
attagacaag aggtctatgt agatagaatt gagttcattc cagttaatcc gacgcgagag 1980
gcgaaagagg atctagaagc agcaaaaaaa gcggtggcga gcttgtttac acgcacaagg 2040
gacggattac aagtaaatgt gaaagattat caagtcgatc aagcggcaaa tttagtgtca 2100
tgcttatcag atgaacaata tgggtatgac aaaaagatgt tattggaagc ggtacgtgcg 2160
gcaaaacgac ttagccgaga acgcaactta cttcaggatc cagattttaa tacaatcaat 2220
agtacagaag aaaatggatg gaaagcaagt aacggcgtta ctattagtga gggcgggcca 2280
ttctataaag gccgtgcaat tcagctagca agtgcacgag aaaattaccc aacatacatc 2340
tatcaaaaag tagatgcatc ggagttaaag ccgtatacac gttatagact ggatgggttc 2400
gtgaaga 2407

<210> 30
<211> 802
<212> PRT
<213> Bacillus thuringiensis

<400> 30
Met Asn Gln Asn Lys His Gly Ile Ile Gly Ala Ser Asn Cys Gly Cys
1 5 10 15

Ala Ser Asp Asp Val Ala Lys Tyr Pro Leu Ala Asn Asn Pro Tyr Ser
20 25 30

Ser Ala Leu Asn Leu Asn Ser Cys Gln Asn Ser Ser Ile Leu Asn Trp
35 40 45

Ile Asn Ile Ile Gly Asp Ala Ala Lys Glu Ala Val Ser Ile Gly Thr
50 55 60

Thr Ile Val Ser Leu Ile Thr Ala Pro Ser Leu Thr Gly Leu Ile Ser
65 70 75 80

Ile Val Tyr Asp Leu Ile Gly Lys Val Leu Gly Gly Ser Ser Gly Gln
85 90 95

Ser Ile Ser Asp Leu Ser Ile Cys Asp Leu Leu Ser Ile Ile Asp Leu
100 105 110

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Arg Val Ser Gln Ser Val Leu Asn Asp Gly Ile Ala Asp Phe Asn Gly
        115                 120                 125

Ser Val Leu Leu Tyr Arg Asn Tyr Leu Glu Ala Leu Asp Ser Trp Asn
    130                 135                 140

Lys Asn Pro Asn Ser Ala Ser Ala Glu Glu Leu Arg Thr Arg Phe Arg
145                 150                 155                 160

Ile Ala Asp Ser Glu Phe Asp Arg Ile Leu Thr Arg Gly Ser Leu Thr
                165                 170                 175

Asn Gly Gly Ser Leu Ala Arg Gln Asn Ala Gln Ile Leu Leu Leu Pro
            180                 185                 190

Ser Phe Ala Ser Ala Ala Phe Phe His Leu Leu Leu Leu Arg Asp Ala
        195                 200                 205

Thr Arg Tyr Gly Thr Asn Trp Gly Leu Tyr Asn Ala Thr Pro Phe Ile
    210                 215                 220

Asn Tyr Gln Ser Lys Leu Val Glu Leu Ile Glu Leu Tyr Thr Asp Tyr
225                 230                 235                 240

Cys Val His Trp Asp Asn Arg Gly Ser Thr Glu Leu Arg Gln Arg Gly
                245                 250                 255

Pro Ser Ala Thr Ala Trp Leu Glu Phe His Arg Tyr Arg Arg Glu Met
            260                 265                 270

Thr Leu Met Gly Leu Glu Ile Val Ala Ser Phe Ser Ser Leu Asp Ile
        275                 280                 285

Thr Asn Tyr Pro Ile Glu Thr Asp Phe Gln Leu Ser Arg Val Ile Tyr
    290                 295                 300

Thr Asp Pro Ile Gly Phe Val His Arg Ser Ser Leu Arg Gly Glu Ser
305                 310                 315                 320

Trp Phe Ser Phe Val Asn Arg Ala Asn Phe Ser Asp Leu Glu Asn Ala
                325                 330                 335

Ile Pro Asn Pro Arg Pro Ser Trp Phe Leu Asn Asn Met Ile Ile Ser
            340                 345                 350

Thr Gly Ser Leu Thr Leu Pro Val Ser Pro Ser Thr Asp Arg Ala Arg
        355                 360                 365

Val Trp Tyr Gly Ser Arg Asp Arg Ile Ser Pro Ala Asn Ser Gln Phe
    370                 375                 380

Ile Thr Glu Leu Ile Ser Gly Gln His Thr Thr Ala Thr Gln Thr Ile
385                 390                 395                 400

Leu Gly Arg Asn Ile Phe Arg Val Asp Ser Gln Ala Cys Asn Leu Asn
                405                 410                 415
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Asp Thr Thr Tyr Gly Val Asn Arg Ala Val Phe Tyr His Asp Ala Ser
420 425 430

Glu Gly Ser Gln Arg Ser Val Tyr Glu Gly Tyr Ile Arg Thr Thr Gly
435 440 445

Ile Asp Asn Pro Arg Val Gln Asn Ile Asn Thr Tyr Leu Pro Gly Glu
450 455 460

Asn Ser Asp Ile Pro Thr Pro Glu Asp Tyr Thr His Ile Leu Ser Thr
465 470 475 480

Thr Ile Asn Leu Thr Gly Gly Leu Arg Gln Val Ala Ser Asn Arg Arg
485 490 495

Ser Ser Leu Val Met Tyr Gly Trp Thr His Lys Ser Leu Ala Arg Asn
500 505 510

Asn Thr Ile Asn Pro Asp Arg Ile Thr Gln Ile Pro Leu Thr Lys Val
515 520 525

Asp Thr Arg Gly Thr Gly Val Ser Tyr Val Asn Asp Pro Gly Phe Ile
530 535 540

Gly Gly Ala Leu Leu Gln Arg Thr Asp His Gly Ser Leu Gly Val Leu
545 550 555 560

Arg Val Gln Phe Pro Leu His Leu Arg Gln Gln Tyr Arg Ile Arg Val
565 570 575

Arg Tyr Ala Ser Thr Thr Asn Ile Arg Leu Ser Val Asn Gly Ser Phe
580 585 590

Gly Thr Ile Ser Gln Asn Leu Pro Ser Thr Met Arg Leu Gly Glu Asp
595 600 605

Leu Arg Tyr Gly Ser Phe Ala Ile Arg Glu Phe Asn Thr Ser Ile Arg
610 615 620

Pro Thr Ala Ser Pro Asp Gln Ile Arg Leu Thr Ile Glu Pro Ser Phe
625 630 635 640

Ile Arg Gln Glu Val Tyr Val Asp Arg Ile Glu Phe Ile Pro Val Asn
645 650 655

Pro Thr Arg Glu Ala Lys Glu Asp Leu Glu Ala Ala Lys Lys Ala Val
660 665 670

Ala Ser Leu Phe Thr Arg Thr Arg Asp Gly Leu Gln Val Asn Val Lys
675 680 685

Asp Tyr Gln Val Asp Gln Ala Ala Asn Leu Val Ser Cys Leu Ser Asp
690 695 700

Glu Gln Tyr Gly Tyr Asp Lys Lys Met Leu Leu Glu Ala Val Arg Ala
705 710 715 720

Ala Lys Arg Leu Ser Arg Glu Arg Asn Leu Leu Gln Asp Pro Asp Phe
725 730 735

Asn Thr Ile Asn Ser Thr Glu Glu Asn Gly Trp Lys Ala Ser Asn Gly
740 745 750

Val Thr Ile Ser Glu Gly Gly Pro Phe Tyr Lys Gly Arg Ala Ile Gln
755 760 765

Leu Ala Ser Ala Arg Glu Asn Tyr Pro Thr Tyr Ile Tyr Gln Lys Val
770 775 780

Asp Ala Ser Glu Leu Lys Pro Tyr Thr Arg Tyr Arg Leu Asp Gly Phe
785 790 795 800

Val Lys

<210> 31
<211> 192
<212> DNA
<213> Bacillus thuringiensis

<400> 31
catttacgca acctcgtatg gatttcattt ccaagaacta tgggaacaga tgacccatta 60
acttctcgtt cgtttgctct tacaactctt ttcacaccaa taaccttaac acgagcacaa 120
gaagaattta atctaacaat accacggggt gtttatatag acagaattga attcgtccca 180
gttatgccac at 192

<210> 32
<211> 64
<212> PRT
<213> Bacillus thuringiensis

<400> 32
His Leu Arg Asn Leu Val Trp Ile Ser Phe Pro Arg Thr Met Gly Thr
1 5 10 15

Asp Asp Pro Leu Thr Ser Arg Ser Phe Ala Leu Thr Thr Leu Phe Thr
20 25 30

Pro Ile Thr Leu Thr Arg Ala Gln Glu Glu Phe Asn Leu Thr Ile Pro
35 40 45

Arg Gly Val Tyr Ile Asp Arg Ile Glu Phe Val Pro Val Met Pro His
50 55 60

<210> 33
<211> 246
<212> DNA

<213> Bacillus thuringiensis

<400> 33
gcttctacta caaatttaca attccataca tcaattgacg gaagacctat taatcagggg 60
aatttttcag caactatgag tagtgggggt aatttacagt ccggaagctt taggactgca 120
ggctttacta ctccgtttaa cttttcaaat ggatcaagta tatttacgtt aagtgctcat 180
gtcttcaatt caggcaatga agtttatata gatcgaattg aatttgttcc ggcagaagta 240
acattt 246

<210> 34
<211> 82
<212> PRT
<213> Bacillus thuringiensis

<400> 34
Ala Ser Thr Thr Asn Leu Gln Phe His Thr Ser Ile Asp Gly Arg Pro
1 5 10 15

Ile Asn Gln Gly Asn Phe Ser Ala Thr Met Ser Ser Gly Gly Asn Leu
20 25 30

Gln Ser Gly Ser Phe Arg Thr Ala Gly Phe Thr Thr Pro Phe Asn Phe
35 40 45

Ser Asn Gly Ser Ser Ile Phe Thr Leu Ser Ala His Val Phe Asn Ser
50 55 60

Gly Asn Glu Val Tyr Ile Asp Arg Ile Glu Phe Val Pro Ala Glu Val
65 70 75 80

Thr Phe

<210> 35
<211> 177
<212> DNA
<213> Bacillus thuringiensis

<400> 35
ctctttccag attatattca gcctcgagtg ttgcagtaac tggaataaat tcaaatctgt 60
ctattatcac tcctgcagtc ccactaaaat ttctaacacc tactatatta cctaatgaag 120
atgtaaaagc attggcactt caaaatcact tgattgtaga ttatctaatg acgtagc 177

<210> 36
<211> 57
<212> PRT
<213> Bacillus thuringiensis

<400> 36
Leu Ser Arg Leu Tyr Ser Ala Ser Ser Val Ala Val Thr Gly Ile Asn
1 5 10 15

Ser Asn Leu Ser Ile Ile Thr Pro Ala Val Pro Leu Lys Phe Leu Thr
20 25 30

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Pro Thr Ile Leu Pro Asn Glu Asp Val Lys Ala Leu Ala Leu Gln Asn
        35                  40                  45

His Leu Ile Val Asp Tyr Leu Met Thr
     50                  55
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<210> 37
<211> 4173
<212> DNA
<213> Bacillus thuringiensis

<220>
<221> CDS
<222> (1)..(3687)

<400> 37

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Met Thr Ser Asn Arg Lys Asn Glu Asn Glu Ile Ile Asn Ala Leu Ser
  1               5                  10                  15

att cca gct gta tcg aat cat tcc aca caa atg gat cta tca cca gat   96
Ile Pro Ala Val Ser Asn His Ser Thr Gln Met Asp Leu Ser Pro Asp
             20                  25                  30

gct cgt att gag gat tct ttg tgt ata gcc gag ggg aat aat atc aat   144
Ala Arg Ile Glu Asp Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asn
         35                  40                  45

cca ctt gtt agc gca tca aca gtc caa acg ggt att aac ata gct ggt   192
Pro Leu Val Ser Ala Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly
     50                  55                  60

aga ata cta ggt gta tta ggc gta ccg ttt gct gga caa ata gct agt   240
Arg Ile Leu Gly Val Leu Gly Val Pro Phe Ala Gly Gln Ile Ala Ser
 65                  70                  75                  80

ttt tat agt ttt ctt gtt ggt gaa tta tgg ccc cgc ggc aga gat cag   288
Phe Tyr Ser Phe Leu Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Gln
                 85                  90                  95

tgg gaa att ttc cta gaa cat gtc gaa caa ctt ata aat caa caa ata   336
Trp Glu Ile Phe Leu Glu His Val Glu Gln Leu Ile Asn Gln Gln Ile
            100                 105                 110

aca gaa aat gct agg aat acg gca ctt gct cga tta caa ggt tta gga   384
Thr Glu Asn Ala Arg Asn Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly
        115                 120                 125

gat tcc ttt aga gcc tat caa cag tca ctt gaa gat tgg cta gaa aac   432
Asp Ser Phe Arg Ala Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn
    130                 135                 140

cgt gat gat gca aga acg aga agt gtt ctt tat acc caa tat ata gcc   480
Arg Asp Asp Ala Arg Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala
145                 150                 155                 160
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tta gaa ctt gat ttt ctt aat gcg atg ccg ctt ttc gca att aga aac   528
Leu Glu Leu Asp Phe Leu Asn Ala Met Pro Leu Phe Ala Ile Arg Asn
                165                 170                 175

caa gaa gtt cca tta tta atg gta tat gct caa gct gca aat tta cac   576
Gln Glu Val Pro Leu Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His
            180                 185                 190

cta tta tta ttg aga gat gcc tct ctt ttt ggt agt gaa ttt ggg ctt   624
Leu Leu Leu Leu Arg Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu
        195                 200                 205

aca tcg cag gaa att caa cgt tat tat gag cgc caa gtg gaa caa acg   672
Thr Ser Gln Glu Ile Gln Arg Tyr Tyr Glu Arg Gln Val Glu Gln Thr
    210                 215                 220

aga gat tat tcc gac tat tgc gta gaa tgg tat aat aca ggt cta aat   720
Arg Asp Tyr Ser Asp Tyr Cys Val Glu Trp Tyr Asn Thr Gly Leu Asn
225                 230                 235                 240

agc ttg aga ggg aca aat gcc gca agt tgg gtg cgt tat aat caa ttc   768
Ser Leu Arg Gly Thr Asn Ala Ala Ser Trp Val Arg Tyr Asn Gln Phe
                245                 250                 255

cgt aga gat cta acg tta ggg gta tta gat cta gtg gca cta ttc cca   816
Arg Arg Asp Leu Thr Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro
            260                 265                 270

agc tat gac act cgc act tat cca ata aat acg agt gct cag tta aca   864
Ser Tyr Asp Thr Arg Thr Tyr Pro Ile Asn Thr Ser Ala Gln Leu Thr
        275                 280                 285

agg gaa gtt tat aca gac gca att gga gca aca ggg gta aat atg gca   912
Arg Glu Val Tyr Thr Asp Ala Ile Gly Ala Thr Gly Val Asn Met Ala
    290                 295                 300

agt atg aat tgg tat aat aat aat gca cct tcg ttt tcc gct ata gag   960
Ser Met Asn Trp Tyr Asn Asn Asn Ala Pro Ser Phe Ser Ala Ile Glu
305                 310                 315                 320

act gcg gtt atc cga agc ccg cat cta ctt gat ttt cta gaa caa ctt  1008
Thr Ala Val Ile Arg Ser Pro His Leu Leu Asp Phe Leu Glu Gln Leu
                325                 330                 335

aca att ttt agc act tca tca cga tgg agt gct act agg cat atg act  1056
Thr Ile Phe Ser Thr Ser Ser Arg Trp Ser Ala Thr Arg His Met Thr
            340                 345                 350

tac tgg cgg ggg cac aca att caa tct cgg cca ata gga ggc gga tta  1104
Tyr Trp Arg Gly His Thr Ile Gln Ser Arg Pro Ile Gly Gly Gly Leu
        355                 360                 365

aat acc tca acg cat ggg tct acc aat act tct att aat cct gta aga  1152
Asn Thr Ser Thr His Gly Ser Thr Asn Thr Ser Ile Asn Pro Val Arg
    370                 375                 380
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tta tca ttc ttc tct cga gac gta tat tgg act gaa tca tat gca gga   1200
Leu Ser Phe Phe Ser Arg Asp Val Tyr Trp Thr Glu Ser Tyr Ala Gly
385                 390                 395                 400

gtg ctt cta tgg gga att tac ctt gaa cct att cat ggt gtc cct act   1248
Val Leu Leu Trp Gly Ile Tyr Leu Glu Pro Ile His Gly Val Pro Thr
                405                 410                 415

gtt aga ttt aat ttt agg aac cct cag aat act ttt gaa aga ggt act   1296
Val Arg Phe Asn Phe Arg Asn Pro Gln Asn Thr Phe Glu Arg Gly Thr
            420                 425                 430

gct aac tat agt caa ccc tat gag tca cct ggg ctt caa tta aaa gat   1344
Ala Asn Tyr Ser Gln Pro Tyr Glu Ser Pro Gly Leu Gln Leu Lys Asp
        435                 440                 445

tca gaa act gaa tta cca cca gaa aca aca gaa cga cca aat tat gaa   1392
Ser Glu Thr Glu Leu Pro Pro Glu Thr Thr Glu Arg Pro Asn Tyr Glu
    450                 455                 460

tca tat agt cat agg tta tct cac ata ggg ctc att tca caa tct agg   1440
Ser Tyr Ser His Arg Leu Ser His Ile Gly Leu Ile Ser Gln Ser Arg
465                 470                 475                 480

gtg cat gta cca gta tat tct tgg acg cac cgt agt gca gat cgt aca   1488
Val His Val Pro Val Tyr Ser Trp Thr His Arg Ser Ala Asp Arg Thr
                485                 490                 495

aat acc att agt tca gat agc ata aca caa ata cca ttg gta aaa tca   1536
Asn Thr Ile Ser Ser Asp Ser Ile Thr Gln Ile Pro Leu Val Lys Ser
            500                 505                 510

ttc aac ctt aat tca ggt acc tct gta gtc agt ggc cca gga ttt aca   1584
Phe Asn Leu Asn Ser Gly Thr Ser Val Val Ser Gly Pro Gly Phe Thr
        515                 520                 525

gga ggg gat ata atc cga act aac gtt aat ggt agt gta cta agt atg   1632
Gly Gly Asp Ile Ile Arg Thr Asn Val Asn Gly Ser Val Leu Ser Met
    530                 535                 540

ggt ctt aat ttt aat aat aca tca tta cag cgg tat cgc gtg aga gtt   1680
Gly Leu Asn Phe Asn Asn Thr Ser Leu Gln Arg Tyr Arg Val Arg Val
545                 550                 555                 560

cgt tat gct gct tct caa aca atg gtc ctg agg gta act gtc gga ggg   1728
Arg Tyr Ala Ala Ser Gln Thr Met Val Leu Arg Val Thr Val Gly Gly
                565                 570                 575

agt act act ttt gat caa gga ttc cct agt act atg agt gca aat gag   1776
Ser Thr Thr Phe Asp Gln Gly Phe Pro Ser Thr Met Ser Ala Asn Glu
            580                 585                 590

tct ttg aca tct caa tca ttt aga ttt gca gaa ttt cct gta ggt att   1824
Ser Leu Thr Ser Gln Ser Phe Arg Phe Ala Glu Phe Pro Val Gly Ile
        595                 600                 605

agt gca tct ggc agt caa act gct gga ata agt ata agt aat aat gca   1872
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Ser Ala Ser Gly Ser Gln Thr Ala Gly Ile Ser Ile Ser Asn Asn Ala
    610                 615                 620

ggt aga caa acg ttt cac ttt gat aaa att gaa ttc att cca att act   1920
Gly Arg Gln Thr Phe His Phe Asp Lys Ile Glu Phe Ile Pro Ile Thr
625                 630                 635                 640

gca acc ttc gaa gca gaa tac gat tta gaa agg gcg caa gag gcg gtg   1968
Ala Thr Phe Glu Ala Glu Tyr Asp Leu Glu Arg Ala Gln Glu Ala Val
                645                 650                 655

aat gct ctg ttt act aat acg aat cca aga aga ttg aaa aca gat gtg   2016
Asn Ala Leu Phe Thr Asn Thr Asn Pro Arg Arg Leu Lys Thr Asp Val
            660                 665                 670

aca gat tat cat att gat caa gta tcc aat tta gtg gcg tgt tta tcg   2064
Thr Asp Tyr His Ile Asp Gln Val Ser Asn Leu Val Ala Cys Leu Ser
        675                 680                 685

gat gaa ttc tgc tta gat gaa aag aga gaa tta ctt gag aaa gtg aaa   2112
Asp Glu Phe Cys Leu Asp Glu Lys Arg Glu Leu Leu Glu Lys Val Lys
    690                 695                 700

tat gcg aaa cga ctc agt gat gaa aga aac tta ctc caa gat cca aac   2160
Tyr Ala Lys Arg Leu Ser Asp Glu Arg Asn Leu Leu Gln Asp Pro Asn
705                 710                 715                 720

ttc aca tcc atc aat aag caa cca gac ttc ata tct act aat gag caa   2208
Phe Thr Ser Ile Asn Lys Gln Pro Asp Phe Ile Ser Thr Asn Glu Gln
                725                 730                 735

tcg aat ttc aca tct atc cat gaa caa tct gaa cat gga tgg tgg gga   2256
Ser Asn Phe Thr Ser Ile His Glu Gln Ser Glu His Gly Trp Trp Gly
            740                 745                 750

agt gag aac att aca atc cag gaa gga aat gac gta ttt aaa gag aat   2304
Ser Glu Asn Ile Thr Ile Gln Glu Gly Asn Asp Val Phe Lys Glu Asn
        755                 760                 765

tac gtc aca cta ccg ggg act ttt aat gag tgt tat ccg acg tat tta   2352
Tyr Val Thr Leu Pro Gly Thr Phe Asn Glu Cys Tyr Pro Thr Tyr Leu
    770                 775                 780

tat caa aaa ata gga gag tcg gaa tta aaa gct tat act cgc tac caa   2400
Tyr Gln Lys Ile Gly Glu Ser Glu Leu Lys Ala Tyr Thr Arg Tyr Gln
785                 790                 795                 800

tta aga ggg tat att gaa gat agt caa gat tta gag ata tat ttg att   2448
Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu Ile Tyr Leu Ile
                805                 810                 815

cgt tat aat gcg aaa cat gaa aca ttg gat gtt cca ggt acc gag tcc   2496
Arg Tyr Asn Ala Lys His Glu Thr Leu Asp Val Pro Gly Thr Glu Ser
            820                 825                 830

gta tgg ccg ctt tca gtt gaa agc cca atc gga agg tgc gga gaa ccg   2544
Val Trp Pro Leu Ser Val Glu Ser Pro Ile Gly Arg Cys Gly Glu Pro
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          835                 840                 845

    aat cga tgc gca cca cat ttt gaa tgg aat cct gat cta gat tgt tcc   2592
    Asn Arg Cys Ala Pro His Phe Glu Trp Asn Pro Asp Leu Asp Cys Ser
5        850                 855                 860

    tgc aga gat gga gaa aaa tgt gcg cat cat tcc cat cat ttc tct ttg   2640
    Cys Arg Asp Gly Glu Lys Cys Ala His His Ser His His Phe Ser Leu
    865                 870                 875                 880
10
    gat att gat att gga tgc aca gac ttg cat gag aat cta ggc gtg tgg   2688
    Asp Ile Asp Ile Gly Cys Thr Asp Leu His Glu Asn Leu Gly Val Trp
                    885                 890                 895

15  gtg gta ttc aag att aag acg cag gaa ggt cat gca aga cta ggg aat   2736
    Val Val Phe Lys Ile Lys Thr Gln Glu Gly His Ala Arg Leu Gly Asn
                900                 905                 910

    ctg gaa ttt att gaa gag aaa cca tta tta gga gaa gca ctg tct cgt   2784
20  Leu Glu Phe Ile Glu Glu Lys Pro Leu Leu Gly Glu Ala Leu Ser Arg
            915                 920                 925

    gtg aag aga gca gag aaa aaa tgg aga gac aaa cgt gaa aaa cta caa   2832
    Val Lys Arg Ala Glu Lys Lys Trp Arg Asp Lys Arg Glu Lys Leu Gln
25      930                 935                 940

    ttg gaa aca aaa cga gta tat aca gag gca aaa gaa gct gtg gat gct   2880
    Leu Glu Thr Lys Arg Val Tyr Thr Glu Ala Lys Glu Ala Val Asp Ala
    945                 950                 955                 960
30
    tta ttt gta gat tct caa tat aat aga tta caa gcg gat aca aac att   2928
    Leu Phe Val Asp Ser Gln Tyr Asn Arg Leu Gln Ala Asp Thr Asn Ile
                    965                 970                 975

35  ggc atg att cat gcg gca gat aaa ctt gtt cat cga att cga gag gct   2976
    Gly Met Ile His Ala Ala Asp Lys Leu Val His Arg Ile Arg Glu Ala
                980                 985                 990

    tat ctg tca gaa tta tct gtt atc ccg ggt gta aat gcg gaa att ttt   3024
40  Tyr Leu Ser Glu Leu Ser Val Ile Pro Gly Val Asn Ala Glu Ile Phe
            995                1000                1005

    gaa gaa tta gaa ggt cgc att atc act gca atc tcc cta tac gat gcg   3072
    Glu Glu Leu Glu Gly Arg Ile Ile Thr Ala Ile Ser Leu Tyr Asp Ala
45      1010                1015                1020

    aga aat gtc gtt aaa aat ggt gat ttt aat aat gga tta gca tgc tgg   3120
    Arg Asn Val Val Lys Asn Gly Asp Phe Asn Asn Gly Leu Ala Cys Trp
    1025                1030                1035                1040
50
    aat gta aaa ggg cat gta gat gta caa cag agc cat cac cgt tct gtc   3168
    Asn Val Lys Gly His Val Asp Val Gln Gln Ser His His Arg Ser Val
                    1045                1050                1055

55  ctt gtt atc cca gaa tgg gaa gca gaa gtg tca caa gca gtt cgc gtc   3216
    Leu Val Ile Pro Glu Trp Glu Ala Glu Val Ser Gln Ala Val Arg Val
                1060                1065                1070
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tgt ccg ggg cgt ggc tat atc ctc cgt gtc aca gcg tac aaa gag gga   3264
Cys Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr Ala Tyr Lys Glu Gly
      1075                1080                1085

tat gga gag ggt tgt gta acg atc cat gaa atc gag aac aat aca gac   3312
Tyr Gly Glu Gly Cys Val Thr Ile His Glu Ile Glu Asn Asn Thr Asp
   1090                1095                1100

gaa cta aaa ttt aaa aac tgt gaa gaa gag gaa gtg tat cca acg gat   3360
Glu Leu Lys Phe Lys Asn Cys Glu Glu Glu Glu Val Tyr Pro Thr Asp
1105                1110                1115                1120

aca gga acg tgt aat gat tat act gca cac caa ggt aca gca gca tgt   3408
Thr Gly Thr Cys Asn Asp Tyr Thr Ala His Gln Gly Thr Ala Ala Cys
                1125                1130                1135

aat tcc cgt aat gct gga tat gag gat gca tat gaa gtt gat act aca   3456
Asn Ser Arg Asn Ala Gly Tyr Glu Asp Ala Tyr Glu Val Asp Thr Thr
            1140                1145                1150

gca tct gtt aat tac aaa ccg act tat gaa gaa gaa acg tat aca gat   3504
Ala Ser Val Asn Tyr Lys Pro Thr Tyr Glu Glu Glu Thr Tyr Thr Asp
      1155                1160                1165

gta cga aga gat aat cat tgt gaa tat gac aga ggg tat gtg aat tat   3552
Val Arg Arg Asp Asn His Cys Glu Tyr Asp Arg Gly Tyr Val Asn Tyr
   1170                1175                1180

cca cca cta cca gct ggt tat gtg aca aag gaa tta gaa tat ttc cca   3600
Pro Pro Leu Pro Ala Gly Tyr Val Thr Lys Glu Leu Glu Tyr Phe Pro
1185                1190                1195                1200

gaa acc gat aag gta tgg att gag att gga gaa acg gaa gga aca ttc   3648
Glu Thr Asp Lys Val Trp Ile Glu Ile Gly Glu Thr Glu Gly Thr Phe
                1205                1210                1215

atc gtg gac agc ata gaa tta ctc ctt atg gaa gaa tag gaccgtccga    3697
Ile Val Asp Ser Ile Glu Leu Leu Leu Met Glu Glu
            1220                1225

gtatagcagt ttaataaatc ttaatcaaaa tagtagtcta acttccgtta caatttaata 3757

agtaaattac agttgtaaaa agaaaacgga catcactcct aagagagcga tgtccgtttt 3817

ctatatggtg tgtgctaacg ataagtgtac acggaatttc attatccaaa ttaatattta 3877

tttgagaaaa ggatcatgtt atatagagat atttccttat aatatttgtt ccacgttcat 3937

aatttttgaa tgatacatta caacaaaaac tgtcacaaat ttatatgttc tacataaaat 3997

atatggttaa gaacctaaga agttatgaat caagtaatag gataaaactg aaaaaggaag 4057

tgtaggtaca atgaataaaa aaataagaaa tgaagatgag cattcatcga tagaattatc 4117

atatagtact tcaaaaaatc aaaagcataa ggtaccattt tgttgtacaa tttcag     4173
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<210> 38
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<212> PRT
<213> Bacillus thuringiensis

<400> 38

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Ile Pro Ala Val Ser Asn His Ser Thr Gln Met Asp Leu Ser Pro Asp
            20                  25                  30
Ala Arg Ile Glu Asp Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asn
        35                  40                  45
Pro Leu Val Ser Ala Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly
    50                  55                  60
Arg Ile Leu Gly Val Leu Gly Val Pro Phe Ala Gly Gln Ile Ala Ser
65                  70                  75                  80
Phe Tyr Ser Phe Leu Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Gln
                85                  90                  95
Trp Glu Ile Phe Leu Glu His Val Glu Gln Leu Ile Asn Gln Gln Ile
            100                 105                 110
Thr Glu Asn Ala Arg Asn Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly
        115                 120                 125
Asp Ser Phe Arg Ala Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn
    130                 135                 140
Arg Asp Asp Ala Arg Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala
145                 150                 155                 160
Leu Glu Leu Asp Phe Leu Asn Ala Met Pro Leu Phe Ala Ile Arg Asn
                165                 170                 175
Gln Glu Val Pro Leu Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His
            180                 185                 190
Leu Leu Leu Leu Arg Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu
        195                 200                 205
Thr Ser Gln Glu Ile Gln Arg Tyr Tyr Glu Arg Gln Val Glu Gln Thr
    210                 215                 220
Arg Asp Tyr Ser Asp Tyr Cys Val Glu Trp Tyr Asn Thr Gly Leu Asn
225                 230                 235                 240
Ser Leu Arg Gly Thr Asn Ala Ala Ser Trp Val Arg Tyr Asn Gln Phe
                245                 250                 255
Arg Arg Asp Leu Thr Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro
            260                 265                 270
Ser Tyr Asp Thr Arg Thr Tyr Pro Ile Asn Thr Ser Ala Gln Leu Thr
        275                 280                 285
Arg Glu Val Tyr Thr Asp Ala Ile Gly Ala Thr Gly Val Asn Met Ala
    290                 295                 300
Ser Met Asn Trp Tyr Asn Asn Asn Ala Pro Ser Phe Ser Ala Ile Glu
305                 310                 315                 320
Thr Ala Val Ile Arg Ser Pro His Leu Leu Asp Phe Leu Glu Gln Leu
                325                 330                 335
Thr Ile Phe Ser Thr Ser Ser Arg Trp Ser Ala Thr Arg His Met Thr
            340                 345                 350
Tyr Trp Arg Gly His Thr Ile Gln Ser Arg Pro Ile Gly Gly Gly Leu
        355                 360                 365
Asn Thr Ser Thr His Gly Ser Thr Asn Thr Ser Ile Asn Pro Val Arg
    370                 375                 380
Leu Ser Phe Phe Ser Arg Asp Val Tyr Trp Thr Glu Ser Tyr Ala Gly
385                 390                 395                 400
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Val Leu Leu Trp Gly Ile Tyr Leu Glu Pro Ile His Gly Val Pro Thr
405 410 415
Val Arg Phe Asn Phe Arg Asn Pro Gln Asn Thr Phe Glu Arg Gly Thr
420 425 430
Ala Asn Tyr Ser Gln Pro Tyr Glu Ser Pro Gly Leu Gln Leu Lys Asp
435 440 445
Ser Glu Thr Glu Leu Pro Pro Glu Thr Thr Glu Arg Pro Asn Tyr Glu
450 455 460
Ser Tyr Ser His Arg Leu Ser His Ile Gly Leu Ile Ser Gln Ser Arg
465 470 475 480
Val His Val Pro Val Tyr Ser Trp Thr His Arg Ser Ala Asp Arg Thr
485 490 495
Asn Thr Ile Ser Ser Asp Ser Ile Thr Gln Ile Pro Leu Val Lys Ser
500 505 510
Phe Asn Leu Asn Ser Gly Thr Ser Val Val Ser Gly Pro Gly Phe Thr
515 520 525
Gly Gly Asp Ile Ile Arg Thr Asn Val Asn Gly Ser Val Leu Ser Met
530 535 540
Gly Leu Asn Phe Asn Asn Thr Ser Leu Gln Arg Tyr Arg Val Arg Val
545 550 555 560
Arg Tyr Ala Ala Ser Gln Thr Met Val Leu Arg Val Thr Val Gly Gly
565 570 575
Ser Thr Thr Phe Asp Gln Gly Phe Pro Ser Thr Met Ser Ala Asn Glu
580 585 590
Ser Leu Thr Ser Gln Ser Phe Arg Phe Ala Glu Phe Pro Val Gly Ile
595 600 605
Ser Ala Ser Gly Ser Gln Thr Ala Gly Ile Ser Ile Ser Asn Asn Ala
610 615 620
Gly Arg Gln Thr Phe His Phe Asp Lys Ile Glu Phe Ile Pro Ile Thr
625 630 635 640
Ala Thr Phe Glu Ala Glu Tyr Asp Leu Glu Arg Ala Gln Glu Ala Val
645 650 655
Asn Ala Leu Phe Thr Asn Thr Asn Pro Arg Arg Leu Lys Thr Asp Val
660 665 670
Thr Asp Tyr His Ile Asp Gln Val Ser Asn Leu Val Ala Cys Leu Ser
675 680 685
Asp Glu Phe Cys Leu Asp Glu Lys Arg Glu Leu Leu Glu Lys Val Lys
690 695 700
Tyr Ala Lys Arg Leu Ser Asp Glu Arg Asn Leu Leu Gln Asp Pro Asn
705 710 715 720
Phe Thr Ser Ile Asn Lys Gln Pro Asp Phe Ile Ser Thr Asn Glu Gln
725 730 735
Ser Asn Phe Thr Ser Ile His Glu Gln Ser Glu His Gly Trp Trp Gly
740 745 750
Ser Glu Asn Ile Thr Ile Gln Glu Gly Asn Asp Val Phe Lys Glu Asn
755 760 765
Tyr Val Thr Leu Pro Gly Thr Phe Asn Glu Cys Tyr Pro Thr Tyr Leu
770 775 780
Tyr Gln Lys Ile Gly Glu Ser Glu Leu Lys Ala Tyr Thr Arg Tyr Gln
785 790 795 800
Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu Ile Tyr Leu Ile
805 810 815
Arg Tyr Asn Ala Lys His Glu Thr Leu Asp Val Pro Gly Thr Glu Ser
820 825 830
Val Trp Pro Leu Ser Val Glu Ser Pro Ile Gly Arg Cys Gly Glu Pro
835 840 845
Asn Arg Cys Ala Pro His Phe Glu Trp Asn Pro Asp Leu Asp Cys Ser

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     850                 855                 860
Cys Arg Asp Gly Glu Lys Cys Ala His His Ser His His Phe Ser Leu
865                 870                 875                 880
Asp Ile Asp Ile Gly Cys Thr Asp Leu His Glu Asn Leu Gly Val Trp
                885                 890                 895
Val Val Phe Lys Ile Lys Thr Gln Glu Gly His Ala Arg Leu Gly Asn
            900                 905                 910
Leu Glu Phe Ile Glu Glu Lys Pro Leu Leu Gly Glu Ala Leu Ser Arg
        915                 920                 925
Val Lys Arg Ala Glu Lys Lys Trp Arg Asp Lys Arg Glu Lys Leu Gln
    930                 935                 940
Leu Glu Thr Lys Arg Val Tyr Thr Glu Ala Lys Glu Ala Val Asp Ala
945                 950                 955                 960
Leu Phe Val Asp Ser Gln Tyr Asn Arg Leu Gln Ala Asp Thr Asn Ile
                965                 970                 975
Gly Met Ile His Ala Ala Asp Lys Leu Val His Arg Ile Arg Glu Ala
            980                 985                 990
Tyr Leu Ser Glu Leu Ser Val Ile Pro Gly Val Asn Ala Glu Ile Phe
        995                1000                1005
Glu Glu Leu Glu Gly Arg Ile Ile Thr Ala Ile Ser Leu Tyr Asp Ala
   1010                1015                1020
Arg Asn Val Val Lys Asn Gly Asp Phe Asn Asn Gly Leu Ala Cys Trp
1025                1030                1035                1040
Asn Val Lys Gly His Val Asp Val Gln Gln Ser His His Arg Ser Val
                1045                1050                1055
Leu Val Ile Pro Glu Trp Glu Ala Glu Val Ser Gln Ala Val Arg Val
            1060                1065                1070
Cys Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr Ala Tyr Lys Glu Gly
        1075                1080                1085
Tyr Gly Glu Gly Cys Val Thr Ile His Glu Ile Glu Asn Asn Thr Asp
   1090                1095                1100
Glu Leu Lys Phe Lys Asn Cys Glu Glu Glu Glu Val Tyr Pro Thr Asp
1105                1110                1115                1120
Thr Gly Thr Cys Asn Asp Tyr Thr Ala His Gln Gly Thr Ala Ala Cys
                1125                1130                1135
Asn Ser Arg Asn Ala Gly Tyr Glu Asp Ala Tyr Glu Val Asp Thr Thr
            1140                1145                1150
Ala Ser Val Asn Tyr Lys Pro Thr Tyr Glu Glu Glu Thr Tyr Thr Asp
        1155                1160                1165
Val Arg Arg Asp Asn His Cys Glu Tyr Asp Arg Gly Tyr Val Asn Tyr
   1170                1175                1180
Pro Pro Leu Pro Ala Gly Tyr Val Thr Lys Glu Leu Glu Tyr Phe Pro
1185                1190                1195                1200
Glu Thr Asp Lys Val Trp Ile Glu Ile Gly Glu Thr Glu Gly Thr Phe
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Ile Val Asp Ser Ile Glu Leu Leu Leu Met Glu Glu
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<220>
<221> CDS

<222> (1)..(3504)

<400> 39

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Met Glu Arg Asn Asn Gln Asp Gln Cys Ile Pro Tyr Asn Cys Leu Asn
 1               5                  10                  15

aat cct gag att gag ata tta gat gtt gaa aat ttc aat ctc gaa ctt   96
Asn Pro Glu Ile Glu Ile Leu Asp Val Glu Asn Phe Asn Leu Glu Leu
            20                  25                  30

gta tcg caa gtc agt gtg gga ctt aca cgt ttt ctt cta gag tca gct  144
Val Ser Gln Val Ser Val Gly Leu Thr Arg Phe Leu Leu Glu Ser Ala
        35                  40                  45

gtc cca gga gcg ggt ttt gca ctt ggc cta ttc gat atc att tgg gga  192
Val Pro Gly Ala Gly Phe Ala Leu Gly Leu Phe Asp Ile Ile Trp Gly
    50                  55                  60

gct cta ggc gtc gat caa tgg agc tta ttc ctt gcg caa att gag caa  240
Ala Leu Gly Val Asp Gln Trp Ser Leu Phe Leu Ala Gln Ile Glu Gln
65                  70                  75                  80

tta att aat gaa agg ata aca aca gtt gaa agg aat aga gca att caa  288
Leu Ile Asn Glu Arg Ile Thr Thr Val Glu Arg Asn Arg Ala Ile Gln
                85                  90                  95

aca tta agt gga cta tcg agt agt tat gaa gta tat att gag gca tta  336
Thr Leu Ser Gly Leu Ser Ser Ser Tyr Glu Val Tyr Ile Glu Ala Leu
            100                 105                 110

aga gaa tgg gag aat aat cca gat aat cca gct tca caa gag aga gtg  384
Arg Glu Trp Glu Asn Asn Pro Asp Asn Pro Ala Ser Gln Glu Arg Val
        115                 120                 125

cgt aca cga ttt cgt aca acg gac gac gct cta ata aca gct ata cct  432
Arg Thr Arg Phe Arg Thr Thr Asp Asp Ala Leu Ile Thr Ala Ile Pro
    130                 135                 140

aat tta gcg att cca gat ttt gag ata gct act tta tca gtg tat gtt  480
Asn Leu Ala Ile Pro Asp Phe Glu Ile Ala Thr Leu Ser Val Tyr Val
145                 150                 155                 160

caa gca gcc aat cta cat cta tct tta tta aga gat gct gtt tac ttt  528
Gln Ala Ala Asn Leu His Leu Ser Leu Leu Arg Asp Ala Val Tyr Phe
                165                 170                 175

gga gaa aga tgg gga ctc aca caa gta aat att gaa gat ctt tat acg  576
Gly Glu Arg Trp Gly Leu Thr Gln Val Asn Ile Glu Asp Leu Tyr Thr
            180                 185                 190

aga tta aca aga aat att cat att tat tca gat cat tgt gca agg tgg  624
Arg Leu Thr Arg Asn Ile His Ile Tyr Ser Asp His Cys Ala Arg Trp
        195                 200                 205

tat aat caa ggt tta aat aat att gga gca aca aat acg aga tat ttg  672
Tyr Asn Gln Gly Leu Asn Asn Ile Gly Ala Thr Asn Thr Arg Tyr Leu
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      210                 215                 220

gaa ttc caa aga gaa tta aca ctc tct gtc tta gat att gtg gcc ctt   720
Glu Phe Gln Arg Glu Leu Thr Leu Ser Val Leu Asp Ile Val Ala Leu
225                 230                 235                 240

ttc ccg aat tac gac atc cga aca tat tca att ccg aca caa agt caa   768
Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Ser Ile Pro Thr Gln Ser Gln
                245                 250                 255

tta aca agg gag att tat acc gat ata att gct gca ccc aat gca tca   816
Leu Thr Arg Glu Ile Tyr Thr Asp Ile Ile Ala Ala Pro Asn Ala Ser
            260                 265                 270

aat tta ata gtg gga acg caa ggc cta gtg aga gca cct cac tta atg   864
Asn Leu Ile Val Gly Thr Gln Gly Leu Val Arg Ala Pro His Leu Met
        275                 280                 285

gac ttt tta gtc cgt ttg aat att tat act ggc ttg gct aga aat att   912
Asp Phe Leu Val Arg Leu Asn Ile Tyr Thr Gly Leu Ala Arg Asn Ile
    290                 295                 300

cgt cat tgg gca gga cat gaa gta ata tct aga aga aca ggt gga gtg   960
Arg His Trp Ala Gly His Glu Val Ile Ser Arg Arg Thr Gly Gly Val
305                 310                 315                 320

gat tta aat act ata caa tct cct tta tat gga aca gct gca act aca   1008
Asp Leu Asn Thr Ile Gln Ser Pro Leu Tyr Gly Thr Ala Ala Thr Thr
                325                 330                 335

gaa agt cca cgt tta ata att cct ttt aat gag gat tct tat ctt ggt   1056
Glu Ser Pro Arg Leu Ile Ile Pro Phe Asn Glu Asp Ser Tyr Leu Gly
            340                 345                 350

ggt ttt att tat aga aca tta tca tcc cct att tat gta cca cct tct   1104
Gly Phe Ile Tyr Arg Thr Leu Ser Ser Pro Ile Tyr Val Pro Pro Ser
        355                 360                 365

gga att tcg agt caa aga aca tct tta gtg gag ggt gtg gga ttt cag   1152
Gly Ile Ser Ser Gln Arg Thr Ser Leu Val Glu Gly Val Gly Phe Gln
    370                 375                 380

aca ccg aat aac tca ata ctt caa tac aga caa cgt gga aca ttg gat   1200
Thr Pro Asn Asn Ser Ile Leu Gln Tyr Arg Gln Arg Gly Thr Leu Asp
385                 390                 395                 400

tcc ctt gag caa gta cca ctt caa gaa gag ggg aga cca ggc ggt ttt   1248
Ser Leu Glu Gln Val Pro Leu Gln Glu Glu Gly Arg Pro Gly Gly Phe
                405                 410                 415

ggt gct agt cat aga ttg tgt cat gct aca ttt gct caa tca cct ata   1296
Gly Ala Ser His Arg Leu Cys His Ala Thr Phe Ala Gln Ser Pro Ile
            420                 425                 430

ggt act aac tat tat ata agg gca ccg ttg ttt tct tgg acg cat ctg   1344
Gly Thr Asn Tyr Tyr Ile Arg Ala Pro Leu Phe Ser Trp Thr His Leu
        435                 440                 445
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agt gca act ctt act aat gaa gtt cgt gta tct aga att aca caa tta   1392
Ser Ala Thr Leu Thr Asn Glu Val Arg Val Ser Arg Ile Thr Gln Leu
    450                 455                 460

ccg atg gtg aag gcg cat acg ctt cat gcg gga gct act gtt gtt aga   1440
Pro Met Val Lys Ala His Thr Leu His Ala Gly Ala Thr Val Val Arg
465                 470                 475                 480

gga cca gga ttt aca gga gga gat ata ctc cga aga act act tca ggc   1488
Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr Thr Ser Gly
                485                 490                 495

tca ttt ggg gat atg aga ata aca aat ttt tca agt tca tca tcg agg   1536
Ser Phe Gly Asp Met Arg Ile Thr Asn Phe Ser Ser Ser Ser Ser Arg
            500                 505                 510

tat cgt gta aga ata cgt tat gct tct act aca gat tta caa ttt ttc   1584
Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr Asp Leu Gln Phe Phe
        515                 520                 525

ttg aat gtt gga gga acc cct gtc aat gta gcc gat ttc ccg aaa acc   1632
Leu Asn Val Gly Gly Thr Pro Val Asn Val Ala Asp Phe Pro Lys Thr
    530                 535                 540

ata gat aga ggg gaa aac tta gaa tat gga agc ttt aga acg gca ggt   1680
Ile Asp Arg Gly Glu Asn Leu Glu Tyr Gly Ser Phe Arg Thr Ala Gly
545                 550                 555                 560

ttt act acc cct ttt agt ttt gta agt tca aca aat aat ttc aca tta   1728
Phe Thr Thr Pro Phe Ser Phe Val Ser Ser Thr Asn Asn Phe Thr Leu
                565                 570                 575

ggt gtt cag agt gtt tct tca ggt aac gag att ttt gta gat cga att   1776
Gly Val Gln Ser Val Ser Ser Gly Asn Glu Ile Phe Val Asp Arg Ile
            580                 585                 590

gaa ttt gtt ccg gca gat gca acc ttt gag gca gaa tat gat tta gaa   1824
Glu Phe Val Pro Ala Asp Ala Thr Phe Glu Ala Glu Tyr Asp Leu Glu
        595                 600                 605

aga gcg caa gag gcg gtg aat gct ctg ttt act tct acg aat caa aga   1872
Arg Ala Gln Glu Ala Val Asn Ala Leu Phe Thr Ser Thr Asn Gln Arg
    610                 615                 620

gga ctg aaa aca gat gtg acg gat tat cat att gat caa gtg tcc aat   1920
Gly Leu Lys Thr Asp Val Thr Asp Tyr His Ile Asp Gln Val Ser Asn
625                 630                 635                 640

tta gtg gat tgt tta tcc gat gaa ttc tgt cta gat gaa aaa aga gaa   1968
Leu Val Asp Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu Lys Arg Glu
                645                 650                 655

ttg tcc gaa aaa att aaa cat gca aag cga ctc agt gat gag cgg aat   2016
Leu Ser Glu Lys Ile Lys His Ala Lys Arg Leu Ser Asp Glu Arg Asn
            660                 665                 670
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tta ctc caa gat tca aac ttt aga ggc atc aat aga caa cca gat cgt   2064
Leu Leu Gln Asp Ser Asn Phe Arg Gly Ile Asn Arg Gln Pro Asp Arg
        675                 680                 685

ggc tgg aga gga agt acg gat att act atc caa gga gga aat gac gta   2112
Gly Trp Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asn Asp Val
    690                 695                 700

ttc aaa gaa aat tac gtc aca cta cca ggt acc ttt gat gag tgc tat   2160
Phe Lys Glu Asn Tyr Val Thr Leu Pro Gly Thr Phe Asp Glu Cys Tyr
705                 710                 715                 720

cca aca tat ttg tat caa aaa atc gat gaa tca aaa tta aaa gcc ttt   2208
Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys Ala Phe
                725                 730                 735

acc cgt tat caa tta aga ggg tat atc gaa gat agt caa gac tta gaa   2256
Thr Arg Tyr Gln Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu
            740                 745                 750

atc tat tta att cgc tac aat gca aaa cat gaa aca gta aat gtg cca   2304
Ile Tyr Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Val Asn Val Pro
        755                 760                 765

ggt acg ggt tcc tta tgg ccg ctt tca gcc caa agt cca atc gga aag   2352
Gly Thr Gly Ser Leu Trp Pro Leu Ser Ala Gln Ser Pro Ile Gly Lys
    770                 775                 780

tgt gga gag ccg aat cga tgc gcg cca cac ctt gaa tgg aat cct gac   2400
Cys Gly Glu Pro Asn Arg Cys Ala Pro His Leu Glu Trp Asn Pro Asp
785                 790                 795                 800

tta gat tgt tcg tgt agg gat gga gaa aag tgt gcc cat cat tcg cat   2448
Leu Asp Cys Ser Cys Arg Asp Gly Glu Lys Cys Ala His His Ser His
                805                 810                 815

cat ttc tcc tta gac att gat gta gga tgt aca gac tta aat gag gac   2496
His Phe Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Asn Glu Asp
            820                 825                 830

cta ggt gta tgg gtg atc ttt aag att aag acg caa gat ggg cac gca   2544
Leu Gly Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp Gly His Ala
        835                 840                 845

aga cta ggg aat cta gag ttt ctc gaa gag aaa cca tta gta gga gaa   2592
Arg Leu Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu Val Gly Glu
    850                 855                 860

gcg cta gct cgt gtg aaa aga gcg gag aaa aaa tgg aga gac aaa cgt   2640
Ala Leu Ala Arg Val Lys Arg Ala Glu Lys Lys Trp Arg Asp Lys Arg
865                 870                 875                 880

gaa aaa ttg gaa tgg gaa aca aat atc gtt tat aaa gag gca aaa gaa   2688
Glu Lys Leu Glu Trp Glu Thr Asn Ile Val Tyr Lys Glu Ala Lys Glu
                885                 890                 895

tct gta gat gct tta ttt gta aac tct caa tat gat caa tta caa gcg   2736
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Ser Val Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Gln Leu Gln Ala
          900                 905                 910

gat acg aat att gcc atg att cat gcg gca gat aaa cgt gtt cat agc   2784
Asp Thr Asn Ile Ala Met Ile His Ala Ala Asp Lys Arg Val His Ser
        915                 920                 925

att cga gaa gct tat ctg cct gag ctg tct gtg att ccg ggt gtc aat   2832
Ile Arg Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly Val Asn
    930                 935                 940

gcg gct att ttt gaa gaa tta gaa ggg cgt att ttc act gca ttc tcc   2880
Ala Ala Ile Phe Glu Glu Leu Glu Gly Arg Ile Phe Thr Ala Phe Ser
945                 950                 955                 960

cta tat gat gcg aga aat gtc att aaa aat ggt gat ttt aat aat ggc   2928
Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn Asn Gly
                965                 970                 975

tta tcc tgc tgg aac gtg aaa ggg cat gta gat gta gaa gaa caa aac   2976
Leu Ser Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu Gln Asn
            980                 985                 990

aac caa cgt tcg gtc ctt gtt gtt ccg gaa tgg gaa gca gaa gtg tca   3024
Asn Gln Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu Val Ser
        995                1000                1005

caa gaa gtt cgt gtc tgt ccg ggt cgt ggc tat atc ctt cgt gtc aca   3072
Gln Glu Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr
    1010                1015                1020

gcg tac aag gag gga tat gga gaa ggt tgc gta acc att cat gag atc   3120
Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile His Glu Ile
1025                1030                1035                1040

gag aac aat aca gac gaa ctg aag ttt agc aac tgc gta gaa gag gaa   3168
Glu Asn Asn Thr Asp Glu Leu Lys Phe Ser Asn Cys Val Glu Glu Glu
                1045                1050                1055

atc tat cca aat aac acg gta acg tgt aat gat tat act gta aat caa   3216
Ile Tyr Pro Asn Asn Thr Val Thr Cys Asn Asp Tyr Thr Val Asn Gln
            1060                1065                1070

gaa gaa tac gga ggt gcg tac act tct cgt aat cga gga tat aac gaa   3264
Glu Glu Tyr Gly Gly Ala Tyr Thr Ser Arg Asn Arg Gly Tyr Asn Glu
        1075                1080                1085

gct cct tcc gta cca gct gat tat gcg tca gtc tat gaa gaa aaa tcg   3312
Ala Pro Ser Val Pro Ala Asp Tyr Ala Ser Val Tyr Glu Glu Lys Ser
    1090                1095                1100

tat aca gat gga cga aga gag aat cct tgt gaa ttt aac aga ggg tat   3360
Tyr Thr Asp Gly Arg Arg Glu Asn Pro Cys Glu Phe Asn Arg Gly Tyr
1105                1110                1115                1120

agg gat tac acg cca cta cca gtt ggt tat gtg aca aaa gaa tta gaa   3408
Arg Asp Tyr Thr Pro Leu Pro Val Gly Tyr Val Thr Lys Glu Leu Glu
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1125 1130 1135

tac ttc cca gaa acc gat aag gta tgg att gag att gga gaa acg gaa 3456
Tyr Phe Pro Glu Thr Asp Lys Val Trp Ile Glu Ile Gly Glu Thr Glu
1140 1145 1150

gga aca ttt atc gtg gac agc gtg gaa tta ctc ctt atg gag gaa tag 3504
Gly Thr Phe Ile Val Asp Ser Val Glu Leu Leu Leu Met Glu Glu
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Val Ser Gln Val Ser Val Gly Leu Thr Arg Phe Leu Leu Glu Ser Ala
35 40 45
Val Pro Gly Ala Gly Phe Ala Leu Gly Leu Phe Asp Ile Ile Trp Gly
50 55 60
Ala Leu Gly Val Asp Gln Trp Ser Leu Phe Leu Ala Gln Ile Glu Gln
65 70 75 80
Leu Ile Asn Glu Arg Ile Thr Thr Val Glu Arg Asn Arg Ala Ile Gln
85 90 95
Thr Leu Ser Gly Leu Ser Ser Ser Tyr Glu Val Tyr Ile Glu Ala Leu
100 105 110
Arg Glu Trp Glu Asn Asn Pro Asp Asn Pro Ala Ser Gln Glu Arg Val
115 120 125
Arg Thr Arg Phe Arg Thr Thr Asp Asp Ala Leu Ile Thr Ala Ile Pro
130 135 140
Asn Leu Ala Ile Pro Asp Phe Glu Ile Ala Thr Leu Ser Val Tyr Val
145 150 155 160
Gln Ala Ala Asn Leu His Leu Ser Leu Leu Arg Asp Ala Val Tyr Phe
165 170 175
Gly Glu Arg Trp Gly Leu Thr Gln Val Asn Ile Glu Asp Leu Tyr Thr
180 185 190
Arg Leu Thr Arg Asn Ile His Ile Tyr Ser Asp His Cys Ala Arg Trp
195 200 205
Tyr Asn Gln Gly Leu Asn Asn Ile Gly Ala Thr Asn Thr Arg Tyr Leu
210 215 220
Glu Phe Gln Arg Glu Leu Thr Leu Ser Val Leu Asp Ile Val Ala Leu
225 230 235 240
Phe Pro Asn Tyr Asp Ile Arg Thr Tyr Ser Ile Pro Thr Gln Ser Gln
245 250 255
Leu Thr Arg Glu Ile Tyr Thr Asp Ile Ile Ala Ala Pro Asn Ala Ser
260 265 270
Asn Leu Ile Val Gly Thr Gln Gly Leu Val Arg Ala Pro His Leu Met
275 280 285
Asp Phe Leu Val Arg Leu Asn Ile Tyr Thr Gly Leu Ala Arg Asn Ile
290 295 300
Arg His Trp Ala Gly His Glu Val Ile Ser Arg Arg Thr Gly Gly Val
305 310 315 320

Asp Leu Asn Thr Ile Gln Ser Pro Leu Tyr Gly Thr Ala Ala Thr Thr
325 330 335
Glu Ser Pro Arg Leu Ile Ile Pro Phe Asn Glu Asp Ser Tyr Leu Gly
340 345 350
Gly Phe Ile Tyr Arg Thr Leu Ser Ser Pro Ile Tyr Val Pro Pro Ser
355 360 365
Gly Ile Ser Ser Gln Arg Thr Ser Leu Val Glu Gly Val Gly Phe Gln
370 375 380
Thr Pro Asn Asn Ser Ile Leu Gln Tyr Arg Gln Arg Gly Thr Leu Asp
385 390 395 400
Ser Leu Glu Gln Val Pro Leu Gln Glu Glu Gly Arg Pro Gly Gly Phe
405 410 415
Gly Ala Ser His Arg Leu Cys His Ala Thr Phe Ala Gln Ser Pro Ile
420 425 430
Gly Thr Asn Tyr Tyr Ile Arg Ala Pro Leu Phe Ser Trp Thr His Leu
435 440 445
Ser Ala Thr Leu Thr Asn Glu Val Arg Val Ser Arg Ile Thr Gln Leu
450 455 460
Pro Met Val Lys Ala His Thr Leu His Ala Gly Ala Thr Val Val Arg
465 470 475 480
Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr Thr Ser Gly
485 490 495
Ser Phe Gly Asp Met Arg Ile Thr Asn Phe Ser Ser Ser Ser Ser Arg
500 505 510
Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr Asp Leu Gln Phe Phe
515 520 525
Leu Asn Val Gly Gly Thr Pro Val Asn Val Ala Asp Phe Pro Lys Thr
530 535 540
Ile Asp Arg Gly Glu Asn Leu Glu Tyr Gly Ser Phe Arg Thr Ala Gly
545 550 555 560
Phe Thr Thr Pro Phe Ser Phe Val Ser Ser Thr Asn Asn Phe Thr Leu
565 570 575
Gly Val Gln Ser Val Ser Ser Gly Asn Glu Ile Phe Val Asp Arg Ile
580 585 590
Glu Phe Val Pro Ala Asp Ala Thr Phe Glu Ala Glu Tyr Asp Leu Glu
595 600 605
Arg Ala Gln Glu Ala Val Asn Ala Leu Phe Thr Ser Thr Asn Gln Arg
610 615 620
Gly Leu Lys Thr Asp Val Thr Asp Tyr His Ile Asp Gln Val Ser Asn
625 630 635 640
Leu Val Asp Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu Lys Arg Glu
645 650 655
Leu Ser Glu Lys Ile Lys His Ala Lys Arg Leu Ser Asp Glu Arg Asn
660 665 670
Leu Leu Gln Asp Ser Asn Phe Arg Gly Ile Asn Arg Gln Pro Asp Arg
675 680 685
Gly Trp Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asn Asp Val
690 695 700
Phe Lys Glu Asn Tyr Val Thr Leu Pro Gly Thr Phe Asp Glu Cys Tyr
705 710 715 720
Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys Ala Phe
725 730 735
Thr Arg Tyr Gln Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu
740 745 750
Ile Tyr Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Val Asn Val Pro
755 760 765
Gly Thr Gly Ser Leu Trp Pro Leu Ser Ala Gln Ser Pro Ile Gly Lys

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     770                 775                 780
Cys Gly Glu Pro Asn Arg Cys Ala Pro His Leu Glu Trp Asn Pro Asp
785                 790                 795                 800
Leu Asp Cys Ser Cys Arg Asp Gly Glu Lys Cys Ala His His Ser His
                805                 810                 815
His Phe Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Asn Glu Asp
            820                 825                 830
Leu Gly Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp Gly His Ala
        835                 840                 845
Arg Leu Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu Val Gly Glu
    850                 855                 860
Ala Leu Ala Arg Val Lys Arg Ala Glu Lys Lys Trp Arg Asp Lys Arg
865                 870                 875                 880
Glu Lys Leu Glu Trp Glu Thr Asn Ile Val Tyr Lys Glu Ala Lys Glu
                885                 890                 895
Ser Val Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Gln Leu Gln Ala
            900                 905                 910
Asp Thr Asn Ile Ala Met Ile His Ala Ala Asp Lys Arg Val His Ser
        915                 920                 925
Ile Arg Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly Val Asn
    930                 935                 940
Ala Ala Ile Phe Glu Glu Leu Glu Gly Arg Ile Phe Thr Ala Phe Ser
945                 950                 955                 960
Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn Asn Gly
                965                 970                 975
Leu Ser Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu Gln Asn
            980                 985                 990
Asn Gln Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu Val Ser
        995                1000                1005
Gln Glu Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr
   1010                1015                1020
Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile His Glu Ile
1025                1030                1035                1040
Glu Asn Asn Thr Asp Glu Leu Lys Phe Ser Asn Cys Val Glu Glu Glu
                1045                1050                1055
Ile Tyr Pro Asn Asn Thr Val Thr Cys Asn Asp Tyr Thr Val Asn Gln
            1060                1065                1070
Glu Glu Tyr Gly Gly Ala Tyr Thr Ser Arg Asn Arg Gly Tyr Asn Glu
        1075                1080                1085
Ala Pro Ser Val Pro Ala Asp Tyr Ala Ser Val Tyr Glu Glu Lys Ser
   1090                1095                1100
Tyr Thr Asp Gly Arg Arg Glu Asn Pro Cys Glu Phe Asn Arg Gly Tyr
1105                1110                1115                1120
Arg Asp Tyr Thr Pro Leu Pro Val Gly Tyr Val Thr Lys Glu Leu Glu
                1125                1130                1135
Tyr Phe Pro Glu Thr Asp Lys Val Trp Ile Glu Ile Gly Glu Thr Glu
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Gly Thr Phe Ile Val Asp Ser Val Glu Leu Leu Leu Met Glu Glu
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  1               5                  10                  15

aca gtt gat aaa aac ttt aca ggt tca cta gaa aat aac aca aat acg   96
Thr Val Asp Lys Asn Phe Thr Gly Ser Leu Glu Asn Asn Thr Asn Thr
            20                  25                  30

gaa tta caa aac ttt aat cat gaa ggt ata gag ccg ttt gtt agt gta  144
Glu Leu Gln Asn Phe Asn His Glu Gly Ile Glu Pro Phe Val Ser Val
        35                  40                  45

tca aca att caa acg ggt att ggt att gct ggt aaa atc ctt ggt aac  192
Ser Thr Ile Gln Thr Gly Ile Gly Ile Ala Gly Lys Ile Leu Gly Asn
    50                  55                  60

cta ggc gtt cct ttt gct ggg caa gta gct agc ctc tat agt ttt atc  240
Leu Gly Val Pro Phe Ala Gly Gln Val Ala Ser Leu Tyr Ser Phe Ile
65                  70                  75                  80

cta ggt gag ctt tgg ccc aaa ggg aaa agc caa tgg gaa atc ttt atg  288
Leu Gly Glu Leu Trp Pro Lys Gly Lys Ser Gln Trp Glu Ile Phe Met
                85                  90                  95

gaa cat gta gaa gag ctt att aat caa aag ata tcg act tat gca aga  336
Glu His Val Glu Glu Leu Ile Asn Gln Lys Ile Ser Thr Tyr Ala Arg
            100                 105                 110

aac aaa gca ctt gca gat tta aaa gga tta gga gat gct ttg gct gtc  384
Asn Lys Ala Leu Ala Asp Leu Lys Gly Leu Gly Asp Ala Leu Ala Val
        115                 120                 125

tac cat gaa tcg ctg gaa agt tgg att gaa aat cgc aat aac aca aga  432
Tyr His Glu Ser Leu Glu Ser Trp Ile Glu Asn Arg Asn Asn Thr Arg
    130                 135                 140

acc aga agt gtt gtc aag agc caa tac atc acc ttg gaa ctt atg ttc  480
Thr Arg Ser Val Val Lys Ser Gln Tyr Ile Thr Leu Glu Leu Met Phe
145                 150                 155                 160

gta caa tca tta cct tct ttt gca gtg tct gga gag gaa gta cca cta  528
Val Gln Ser Leu Pro Ser Phe Ala Val Ser Gly Glu Glu Val Pro Leu
                165                 170                 175

tta cca ata tat gct caa gct gca aat tta cac tta ttg cta tta cga  576
Leu Pro Ile Tyr Ala Gln Ala Ala Asn Leu His Leu Leu Leu Leu Arg
            180                 185                 190

gat gct tct att ttt gga aaa taa tgg ggg tta tca gac tca gaa att  624
Asp Ala Ser Ile Phe Gly Lys Xaa Trp Gly Leu Ser Asp Ser Glu Ile
        195                 200                 205
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tcc aca ttt tat aat cgc caa tcc gga aaa tcg aaa gaa tat tct gac   672
Ser Thr Phe Tyr Asn Arg Gln Ser Gly Lys Ser Lys Glu Tyr Ser Asp
    210                 215                 220

cac tgc gta aaa tgg tat aat aca ggc cta aat cgc ttg atg ggg aac   720
His Cys Val Lys Trp Tyr Asn Thr Gly Leu Asn Arg Leu Met Gly Asn
225                 230                 235                 240

aat gcc gaa agt tgg gta cga tat aat caa ttc cgt aga gac atg act   768
Asn Ala Glu Ser Trp Val Arg Tyr Asn Gln Phe Arg Arg Asp Met Thr
                245                 250                 255

tta atg gta cta gat tta gtg gca cta ttt cca agc tat gat aca caa   816
Leu Met Val Leu Asp Leu Val Ala Leu Phe Pro Ser Tyr Asp Thr Gln
            260                 265                 270

atg tat cca att aaa act aca gcc caa ctt aca aga gaa gta tat aca   864
Met Tyr Pro Ile Lys Thr Thr Ala Gln Leu Thr Arg Glu Val Tyr Thr
        275                 280                 285

gac gca att ggg aca gta cat ccg cat cca agt ttt aca agt acg act   912
Asp Ala Ile Gly Thr Val His Pro His Pro Ser Phe Thr Ser Thr Thr
    290                 295                 300

tgg tat aat aat aat gca cct tcg ttc tct acc ata gag gct gct gtt   960
Trp Tyr Asn Asn Asn Ala Pro Ser Phe Ser Thr Ile Glu Ala Ala Val
305                 310                 315                 320

gtt cga aac ccg cat cta ctc gat ttt cta gaa caa gtt aca att tac   1008
Val Arg Asn Pro His Leu Leu Asp Phe Leu Glu Gln Val Thr Ile Tyr
                325                 330                 335

agc tta tta agt cga tgg agt aac act cag tat atg aat atg tgg gga   1056
Ser Leu Leu Ser Arg Trp Ser Asn Thr Gln Tyr Met Asn Met Trp Gly
            340                 345                 350

gga cat aaa cta gaa ttc cga aca ata gga gga acg tta aat acc tca   1104
Gly His Lys Leu Glu Phe Arg Thr Ile Gly Gly Thr Leu Asn Thr Ser
        355                 360                 365

aca caa gga tct act aat act tct att aat cct gta aca tta ccg ttc   1152
Thr Gln Gly Ser Thr Asn Thr Ser Ile Asn Pro Val Thr Leu Pro Phe
    370                 375                 380

act tct cga gac gtc tat agg act gaa tca ttg gca ggg ctg aat cta   1200
Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Leu Ala Gly Leu Asn Leu
385                 390                 395                 400

ttt tta act caa cct gtt aat gga gta cct agg gtt gat ttt cat tgg   1248
Phe Leu Thr Gln Pro Val Asn Gly Val Pro Arg Val Asp Phe His Trp
                405                 410                 415

aaa ttc gtc aca cat ccg atc gca tct gat aat ttc tat tat cca ggg   1296
Lys Phe Val Thr His Pro Ile Ala Ser Asp Asn Phe Tyr Tyr Pro Gly
            420                 425                 430

tat gct gga att ggg acg caa tta cag gat tca gaa aat gaa tta cca   1344
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Tyr Ala Gly Ile Gly Thr Gln Leu Gln Asp Ser Glu Asn Glu Leu Pro
        435                 440                 445

cct gaa gca aca gga cag cca aat tat gaa tct tat agt cat aga tta   1392
Pro Glu Ala Thr Gly Gln Pro Asn Tyr Glu Ser Tyr Ser His Arg Leu
    450                 455                 460

tct cat ata gga ctc att tca gca tca cat gtg aaa gca ttg gta tat   1440
Ser His Ile Gly Leu Ile Ser Ala Ser His Val Lys Ala Leu Val Tyr
465                 470                 475                 480

tct tgg acg cat cgt agt gca gat cgt aca aat aca att gag cca aat   1488
Ser Trp Thr His Arg Ser Ala Asp Arg Thr Asn Thr Ile Glu Pro Asn
                485                 490                 495

agc att aca caa ata cca tta gta aaa gcg ttc aat ctg tct tca ggt   1536
Ser Ile Thr Gln Ile Pro Leu Val Lys Ala Phe Asn Leu Ser Ser Gly
            500                 505                 510

gcc gct gta gtg aga gga cca gga ttt aca ggt ggg gat atc ctt cga   1584
Ala Ala Val Val Arg Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu Arg
        515                 520                 525

aga aag aat act ggt aca ttt ggg gat ata cga gta aat att aat cca   1632
Arg Lys Asn Thr Gly Thr Phe Gly Asp Ile Arg Val Asn Ile Asn Pro
    530                 535                 540

cca ttt gca caa aga tat cgc gtg agg att cgc tat gct tct acc aca   1680
Pro Phe Ala Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
545                 550                 555                 560

gat tta caa ttc cat acg tca att aac ggt aaa gct att aat caa ggt   1728
Asp Leu Gln Phe His Thr Ser Ile Asn Gly Lys Ala Ile Asn Gln Gly
                565                 570                 575

aat ttt tca gca act atg aat aga gga gag gac tta gac tat aaa acc   1776
Asn Phe Ser Ala Thr Met Asn Arg Gly Glu Asp Leu Asp Tyr Lys Thr
            580                 585                 590

ttt aga act gta ggc ttt acc acc cca ttt agc ttt tca gat gta caa   1824
Phe Arg Thr Val Gly Phe Thr Thr Pro Phe Ser Phe Ser Asp Val Gln
        595                 600                 605

agt aca ttc aca ata ggt gct tgg aac ttc tct tca ggt aac gaa gtt   1872
Ser Thr Phe Thr Ile Gly Ala Trp Asn Phe Ser Ser Gly Asn Glu Val
    610                 615                 620

tat ata gat aga att gaa ttt gtt ccg gta gaa gta aca tat gag gca   1920
Tyr Ile Asp Arg Ile Glu Phe Val Pro Val Glu Val Thr Tyr Glu Ala
625                 630                 635                 640

gaa tat gat ttt gaa aaa gcg caa gag gag gtt act gca ctg ttt aca   1968
Glu Tyr Asp Phe Glu Lys Ala Gln Glu Glu Val Thr Ala Leu Phe Thr
                645                 650                 655

tct acg aat cca aga gga tta aaa aca gat gta aag gat tat cat att   2016
Ser Thr Asn Pro Arg Gly Leu Lys Thr Asp Val Lys Asp Tyr His Ile
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660 665 670

gac cag gta tca aat tta gta gag tct cta tca gat aaa ttc tat ctt 2064
Asp Gln Val Ser Asn Leu Val Glu Ser Leu Ser Asp Lys Phe Tyr Leu
675 680 685

gat gaa aag aga gaa tta ttc gag ata gtt aaa tac gcg aag caa ctc 2112
Asp Glu Lys Arg Glu Leu Phe Glu Ile Val Lys Tyr Ala Lys Gln Leu
690 695 700

cat att gag cgt aac atg tag 2133
His Ile Glu Arg Asn Met
705 710

<210> 42
<211> 710
<212> PRT
<213> Bacillus thuringiensis

<400> 42
Met Lys Ser Lys Asn Gln Asn Met His Gln Ser Leu Ser Asn Asn Ala
1 5 10 15
Thr Val Asp Lys Asn Phe Thr Gly Ser Leu Glu Asn Asn Thr Asn Thr
20 25 30
Glu Leu Gln Asn Phe Asn His Glu Gly Ile Glu Pro Phe Val Ser Val
35 40 45
Ser Thr Ile Gln Thr Gly Ile Gly Ile Ala Gly Lys Ile Leu Gly Asn
50 55 60
Leu Gly Val Pro Phe Ala Gly Gln Val Ala Ser Leu Tyr Ser Phe Ile
65 70 75 80
Leu Gly Glu Leu Trp Pro Lys Gly Lys Ser Gln Trp Glu Ile Phe Met
85 90 95
Glu His Val Glu Glu Leu Ile Asn Gln Lys Ile Ser Thr Tyr Ala Arg
100 105 110
Asn Lys Ala Leu Ala Asp Leu Lys Gly Leu Gly Asp Ala Leu Ala Val
115 120 125
Tyr His Glu Ser Leu Glu Ser Trp Ile Glu Asn Arg Asn Asn Thr Arg
130 135 140
Thr Arg Ser Val Val Lys Ser Gln Tyr Ile Thr Leu Glu Leu Met Phe
145 150 155 160
Val Gln Ser Leu Pro Ser Phe Ala Val Ser Gly Glu Glu Val Pro Leu
165 170 175
Leu Pro Ile Tyr Ala Gln Ala Ala Asn Leu His Leu Leu Leu Leu Arg
180 185 190
Asp Ala Ser Ile Phe Gly Lys Xaa Trp Gly Leu Ser Asp Ser Glu Ile
195 200 205
Ser Thr Phe Tyr Asn Arg Gln Ser Gly Lys Ser Lys Glu Tyr Ser Asp
210 215 220
His Cys Val Lys Trp Tyr Asn Thr Gly Leu Asn Arg Leu Met Gly Asn
225 230 235 240
Asn Ala Glu Ser Trp Val Arg Tyr Asn Gln Phe Arg Arg Asp Met Thr
245 250 255
Leu Met Val Leu Asp Leu Val Ala Leu Phe Pro Ser Tyr Asp Thr Gln
260 265 270
Met Tyr Pro Ile Lys Thr Thr Ala Gln Leu Thr Arg Glu Val Tyr Thr
275 280 285

Asp Ala Ile Gly Thr Val His Pro His Pro Ser Phe Thr Ser Thr Thr
290 295 300
Trp Tyr Asn Asn Asn Ala Pro Ser Phe Ser Thr Ile Glu Ala Ala Val
305 310 315 320
Val Arg Asn Pro His Leu Leu Asp Phe Leu Glu Gln Val Thr Ile Tyr
325 330 335
Ser Leu Leu Ser Arg Trp Ser Asn Thr Gln Tyr Met Asn Met Trp Gly
340 345 350
Gly His Lys Leu Glu Phe Arg Thr Ile Gly Gly Thr Leu Asn Thr Ser
355 360 365
Thr Gln Gly Ser Thr Asn Thr Ser Ile Asn Pro Val Thr Leu Pro Phe
370 375 380
Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Leu Ala Gly Leu Asn Leu
385 390 395 400
Phe Leu Thr Gln Pro Val Asn Gly Val Pro Arg Val Asp Phe His Trp
405 410 415
Lys Phe Val Thr His Pro Ile Ala Ser Asp Asn Phe Tyr Tyr Pro Gly
420 425 430
Tyr Ala Gly Ile Gly Thr Gln Leu Gln Asp Ser Glu Asn Glu Leu Pro
435 440 445
Pro Glu Ala Thr Gly Gln Pro Asn Tyr Glu Ser Tyr Ser His Arg Leu
450 455 460
Ser His Ile Gly Leu Ile Ser Ala Ser His Val Lys Ala Leu Val Tyr
465 470 475 480
Ser Trp Thr His Arg Ser Ala Asp Arg Thr Asn Thr Ile Glu Pro Asn
485 490 495
Ser Ile Thr Gln Ile Pro Leu Val Lys Ala Phe Asn Leu Ser Ser Gly
500 505 510
Ala Ala Val Val Arg Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu Arg
515 520 525
Arg Lys Asn Thr Gly Thr Phe Gly Asp Ile Arg Val Asn Ile Asn Pro
530 535 540
Pro Phe Ala Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
545 550 555 560
Asp Leu Gln Phe His Thr Ser Ile Asn Gly Lys Ala Ile Asn Gln Gly
565 570 575
Asn Phe Ser Ala Thr Met Asn Arg Gly Glu Asp Leu Asp Tyr Lys Thr
580 585 590
Phe Arg Thr Val Gly Phe Thr Thr Pro Phe Ser Phe Ser Asp Val Gln
595 600 605
Ser Thr Phe Thr Ile Gly Ala Trp Asn Phe Ser Ser Gly Asn Glu Val
610 615 620
Tyr Ile Asp Arg Ile Glu Phe Val Pro Val Glu Val Thr Tyr Glu Ala
625 630 635 640
Glu Tyr Asp Phe Glu Lys Ala Gln Glu Glu Val Thr Ala Leu Phe Thr
645 650 655
Ser Thr Asn Pro Arg Gly Leu Lys Thr Asp Val Lys Asp Tyr His Ile
660 665 670
Asp Gln Val Ser Asn Leu Val Glu Ser Leu Ser Asp Lys Phe Tyr Leu
675 680 685
Asp Glu Lys Arg Glu Leu Phe Glu Ile Val Lys Tyr Ala Lys Gln Leu
690 695 700
His Ile Glu Arg Asn Met
705 710

<210> 43
<211> 218
<212> DNA
<213> Bacillus thuringiensis

<400> 43
gtagccgatt tcccgaaaac catagataga ggggaaaact tagaatatgg aagctttaga 60
acggcaggtt ttactacccc ttttagtttt gtaagttcaa caaataattt cacattaggt 120
gttcagagtg tttcttcagg taacgagatt tttgtagatc gaattgaatt tgttccggca 180
gatgcaacct ttgaggcaga atatgattta gaaagagc 218

<210> 44
<211> 72
<212> PRT
<213> Bacillus thuringiensis

<400> 44
Val Ala Asp Phe Pro Lys Thr Ile Asp Arg Gly Glu Asn Leu Glu Tyr
1 5 10 15

Gly Ser Phe Arg Thr Ala Gly Phe Thr Thr Pro Phe Ser Phe Val Ser
20 25 30

Ser Thr Asn Asn Phe Thr Leu Gly Val Gln Ser Val Ser Ser Gly Asn
35 40 45

Glu Ile Phe Val Asp Arg Ile Glu Phe Val Pro Ala Asp Ala Thr Phe
50 55 60

Glu Ala Glu Tyr Asp Leu Glu Arg
65 70

<210> 45
<211> 1908
<212> DNA
<213> Bacillus thuringiensis

<220>
<221> CDS
<222> (1)..(1908)

<400> 45
atg aat aat gta ttg aat agc gga aaa aca act att tgt aat gcg tat 48
Met Asn Asn Val Leu Asn Ser Gly Lys Thr Thr Ile Cys Asn Ala Tyr
1 5 10 15

aat gta gtg gct cac gat cca ttt agt ttt gaa cat aaa tca tta gat 96
Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asp
20 25 30

acc atc caa gaa gaa tgg atg gag tgg aaa aga aca gat cat agt tta 144
Thr Ile Gln Glu Glu Trp Met Glu Trp Lys Arg Thr Asp His Ser Leu
35 40 45

tat gta gct cct gta gtc gga act gtg tct agt ttt ctg cta aag aaa 192

```
Tyr Val Ala Pro Val Val Gly Thr Val Ser Ser Phe Leu Leu Lys Lys
     50                  55                  60

gtg ggg agt cta att gga aaa agg ata ttg agt gaa tta tgg ggg tta   240
Val Gly Ser Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Trp Gly Leu
 65                  70                  75                  80

ata ttt cct agt ggt agt aca aat cta atg caa gat att tta aga gag   288
Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu
                 85                  90                  95

aca gaa caa ttc cta aat caa aga ctt aat aca gac acc ctt gat cgt   336
Thr Glu Gln Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Leu Asp Arg
            100                 105                 110

gta aat gca gaa ttg gaa ggg ctc caa gcg aat ata agg gag ttt aat   384
Val Asn Ala Glu Leu Glu Gly Leu Gln Ala Asn Ile Arg Glu Phe Asn
        115                 120                 125

caa caa gta gat aat ttt tta aac cct act caa aac cct gtt cct tta   432
Gln Gln Val Asp Asn Phe Leu Asn Pro Thr Gln Asn Pro Val Pro Leu
    130                 135                 140

tca ata act tct tca gtt aat aca atg cag caa tta ttt cta aat aga   480
Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg
145                 150                 155                 160

tta ccc cag ttc cag ata caa gga tac cag ttg tta tta tta cct tta   528
Leu Pro Gln Phe Gln Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
                165                 170                 175

ttt gca cag gca gcc aat atg cat ctt tct ttt att aga gat gtt att   576
Phe Ala Gln Ala Ala Asn Met His Leu Ser Phe Ile Arg Asp Val Ile
            180                 185                 190

ctt aat gca gat gaa tgg ggc att tca gca gca aca cta cgt acg tat   624
Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr
        195                 200                 205

cga gac tac ctg aga aat tat aca aga gat tat tct aat tat tgt ata   672
Arg Asp Tyr Leu Arg Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile
    210                 215                 220

aat acg tat caa act gcg ttt aga ggg tta aac acc cgt tta cac gat   720
Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp
225                 230                 235                 240

atg tta gaa ttt aga aca tat atg ttt tta aat gta ttt gaa tat gta   768
Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val
                245                 250                 255

tcc att tgg tca ttg ttt aaa tat cag agt ctt atg gta tct tct ggc   816
Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Met Val Ser Ser Gly
            260                 265                 270

gct aat tta tat gct agt ggt agt gga cca cag cag aca caa tca ttt   864
Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Ser Phe
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          275                 280                 285

    act gca caa aac tgg cca ttt tta tat tct ctt ttc caa gtt aat tcg   912
    Thr Ala Gln Asn Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser
 5      290                 295                 300

    aat tat ata tta tct ggt att agt ggt aat agg ctt tct act acc ttc   960
    Asn Tyr Ile Leu Ser Gly Ile Ser Gly Asn Arg Leu Ser Thr Thr Phe
    305                 310                 315                 320
10
    cct aat att ggt ggt tta ccg ggt agt act aca att cat tca ttg aac   1008
    Pro Asn Ile Gly Gly Leu Pro Gly Ser Thr Thr Ile His Ser Leu Asn
                    325                 330                 335

15  agt gcc agg gtt aat tat agc gga gga gtt tca tct ggt ctc ata ggg   1056
    Ser Ala Arg Val Asn Tyr Ser Gly Gly Val Ser Ser Gly Leu Ile Gly
                340                 345                 350

    gcg act aat ctc aat cac aac ttt aat tgc agc acg gtc ctc cct cct   1104
20  Ala Thr Asn Leu Asn His Asn Phe Asn Cys Ser Thr Val Leu Pro Pro
            355                 360                 365

    tta tca aca cca ttt gtt aga agt tgg ctg gat tca ggt aca gat cga   1152
    Leu Ser Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Thr Asp Arg
25      370                 375                 380

    gag ggc gtt gct acc tct acg act tgg cag aca gaa tcc ttc caa ata   1200
    Glu Gly Val Ala Thr Ser Thr Thr Trp Gln Thr Glu Ser Phe Gln Ile
    385                 390                 395                 400
30
    act tca ggt tta agg tgt ggt gct ttt cct ttt tca gct cgt gga aat   1248
    Thr Ser Gly Leu Arg Cys Gly Ala Phe Pro Phe Ser Ala Arg Gly Asn
                    405                 410                 415

35  tca aac tat ttc cca gat tat ttt atc cgt aat att tct ggg gtt cct   1296
    Ser Asn Tyr Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro
                420                 425                 430

    tta gtt att aga aac gaa gat cta aca aga ccg tta cac tat aac caa   1344
40  Leu Val Ile Arg Asn Glu Asp Leu Thr Arg Pro Leu His Tyr Asn Gln
            435                 440                 445

    ata aga aat ata gaa agt cct tcg gga aca cct ggt gga tta cga gct   1392
    Ile Arg Asn Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Leu Arg Ala
45      450                 455                 460

    tat atg gta tct gtg cat aac aga aaa aat aat atc tat gcc gct cat   1440
    Tyr Met Val Ser Val His Asn Arg Lys Asn Asn Ile Tyr Ala Ala His
    465                 470                 475                 480
50
    gaa aat ggt act atg att cat ttg gca ccg gaa gat tat aca gga ttt   1488
    Glu Asn Gly Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe
                    485                 490                 495

55  act ata tca cca ata cat gcc act caa gtg aat aat caa act cga aca   1536
    Thr Ile Ser Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr
                500                 505                 510
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ttt att tct gaa aaa ttt gga aat caa ggt gat tcc tta aga ttt gaa   1584
Phe Ile Ser Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu
        515                 520                 525

caa agt aac acg aca gct cgt tat acg ctt aga ggg aat gga aat agt   1632
Gln Ser Asn Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser
    530                 535                 540

tac aat ctt tat tta aga gta tct tca ata gga aat tca act atc cga   1680
Tyr Asn Leu Tyr Leu Arg Val Ser Ser Ile Gly Asn Ser Thr Ile Arg
545                 550                 555                 560

gtt act ata aac ggt agg gtt tat act gct tca aat gtt aat act aat   1728
Val Thr Ile Asn Gly Arg Val Tyr Thr Ala Ser Asn Val Asn Thr Asn
                565                 570                 575

aca aat aac gat ggg gtt aat gat aat gga gct cgt ttt tca gat att   1776
Thr Asn Asn Asp Gly Val Asn Asp Asn Gly Ala Arg Phe Ser Asp Ile
            580                 585                 590

aat atc ggt aat gta gta gca agt gat aat act aat gta ccg tta gat   1824
Asn Ile Gly Asn Val Val Ala Ser Asp Asn Thr Asn Val Pro Leu Asp
        595                 600                 605

ata aat gtg aca tta aac tcc ggt act caa ttt gag ctt atg aat att   1872
Ile Asn Val Thr Leu Asn Ser Gly Thr Gln Phe Glu Leu Met Asn Ile
    610                 615                 620

atg ttt gtg cca act aat ctt cca cca ctt tat taa                  1908
Met Phe Val Pro Thr Asn Leu Pro Pro Leu Tyr
625                 630                 635
```

<210> 46
<211> 635
<212> PRT
<213> Bacillus thuringiensis

<400> 46

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Met Asn Asn Val Leu Asn Ser Gly Lys Thr Thr Ile Cys Asn Ala Tyr
  1               5                  10                  15
Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asp
            20                  25                  30
Thr Ile Gln Glu Glu Trp Met Glu Trp Lys Arg Thr Asp His Ser Leu
        35                  40                  45
Tyr Val Ala Pro Val Val Gly Thr Val Ser Ser Phe Leu Leu Lys Lys
    50                  55                  60
Val Gly Ser Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Trp Gly Leu
65                  70                  75                  80
Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu
                85                  90                  95
Thr Glu Gln Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Leu Asp Arg
            100                 105                 110
Val Asn Ala Glu Leu Glu Gly Leu Gln Ala Asn Ile Arg Glu Phe Asn
        115                 120                 125
Gln Gln Val Asp Asn Phe Leu Asn Pro Thr Gln Asn Pro Val Pro Leu
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    130                 135                 140
Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg
145                 150                 155                 160
Leu Pro Gln Phe Gln Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
                165                 170                 175
Phe Ala Gln Ala Ala Asn Met His Leu Ser Phe Ile Arg Asp Val Ile
            180                 185                 190
Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr
        195                 200                 205
Arg Asp Tyr Leu Arg Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile
    210                 215                 220
Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp
225                 230                 235                 240
Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val
                245                 250                 255
Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Met Val Ser Ser Gly
            260                 265                 270
Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Ser Phe
        275                 280                 285
Thr Ala Gln Asn Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser
    290                 295                 300
Asn Tyr Ile Leu Ser Gly Ile Ser Gly Asn Arg Leu Ser Thr Thr Phe
305                 310                 315                 320
Pro Asn Ile Gly Gly Leu Pro Gly Ser Thr Thr Ile His Ser Leu Asn
                325                 330                 335
Ser Ala Arg Val Asn Tyr Ser Gly Gly Val Ser Ser Gly Leu Ile Gly
            340                 345                 350
Ala Thr Asn Leu Asn His Asn Phe Asn Cys Ser Thr Val Leu Pro Pro
        355                 360                 365
Leu Ser Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Thr Asp Arg
    370                 375                 380
Glu Gly Val Ala Thr Ser Thr Thr Trp Gln Thr Glu Ser Phe Gln Ile
385                 390                 395                 400
Thr Ser Gly Leu Arg Cys Gly Ala Phe Pro Phe Ser Ala Arg Gly Asn
                405                 410                 415
Ser Asn Tyr Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro
            420                 425                 430
Leu Val Ile Arg Asn Glu Asp Leu Thr Arg Pro Leu His Tyr Asn Gln
        435                 440                 445
Ile Arg Asn Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Leu Arg Ala
    450                 455                 460
Tyr Met Val Ser Val His Asn Arg Lys Asn Asn Ile Tyr Ala Ala His
465                 470                 475                 480
Glu Asn Gly Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe
                485                 490                 495
Thr Ile Ser Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr
            500                 505                 510
Phe Ile Ser Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu
        515                 520                 525
Gln Ser Asn Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser
    530                 535                 540
Tyr Asn Leu Tyr Leu Arg Val Ser Ser Ile Gly Asn Ser Thr Ile Arg
545                 550                 555                 560
Val Thr Ile Asn Gly Arg Val Tyr Thr Ala Ser Asn Val Asn Thr Asn
                565                 570                 575
Thr Asn Asn Asp Gly Val Asn Asp Asn Gly Ala Arg Phe Ser Asp Ile
            580                 585                 590
```

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Asn Ile Gly Asn Val Val Ala Ser Asp Asn Thr Asn Val Pro Leu Asp
        595                 600                 605
Ile Asn Val Thr Leu Asn Ser Gly Thr Gln Phe Glu Leu Met Asn Ile
    610                 615                 620
Met Phe Val Pro Thr Asn Leu Pro Pro Leu Tyr
625                 630                 635
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<210> 47
<211> 1878
<212> DNA
<213> Bacillus thuringiensis

<220>
<221> CDS
<222> (1)..(1878)

<400> 47

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atg aat act gta ttg aat aac gga aga aat act act tgt cat gca cat   48
Met Asn Thr Val Leu Asn Asn Gly Arg Asn Thr Thr Cys His Ala His
  1               5                  10                  15

aat gta gtt gct cat gat cca ttt agt ttt gaa cat aaa tca tta aat   96
Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asn
            20                  25                  30

acc ata gaa aaa gaa tgg aaa gaa tgg aaa aga act gat cat agt tta   144
Thr Ile Glu Lys Glu Trp Lys Glu Trp Lys Arg Thr Asp His Ser Leu
        35                  40                  45

tat gta gcc cct att gtg gga act gtg ggt agt ttt cta tta aag aaa   192
Tyr Val Ala Pro Ile Val Gly Thr Val Gly Ser Phe Leu Leu Lys Lys
    50                  55                  60

gta ggg agt ctt gtt gga aaa agg ata ctg agt gag tta cag aat tta   240
Val Gly Ser Leu Val Gly Lys Arg Ile Leu Ser Glu Leu Gln Asn Leu
65                  70                  75                  80

att ttt cct agt ggt agt ata gat tta atg caa gag att tta aga gcg   288
Ile Phe Pro Ser Gly Ser Ile Asp Leu Met Gln Glu Ile Leu Arg Ala
                85                  90                  95

aca gaa caa ttc ata aat caa agg ctt aat gca gac acc ctt ggt cgt   336
Thr Glu Gln Phe Ile Asn Gln Arg Leu Asn Ala Asp Thr Leu Gly Arg
            100                 105                 110

gta aat gca gaa ttg gca ggt ctt caa gcg aat gtg gca gag ttt aat   384
Val Asn Ala Glu Leu Ala Gly Leu Gln Ala Asn Val Ala Glu Phe Asn
        115                 120                 125

cga caa gta gat aat ttt tta aac cct aat caa aac cct gtt cct tta   432
Arg Gln Val Asp Asn Phe Leu Asn Pro Asn Gln Asn Pro Val Pro Leu
    130                 135                 140

gca ata att gat tca gtt aat aca ttg cag caa tta ttt cta agt aga   480
Ala Ile Ile Asp Ser Val Asn Thr Leu Gln Gln Leu Phe Leu Ser Arg
```

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145                 150                 155                 160

tta cca cag ttc cag ata caa ggc tat caa ctg tta tta tta cct tta   528
Leu Pro Gln Phe Gln Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
                165                 170                 175

ttt gca cag gca gcc aat tta cat ctt tct ttt att aga gat gtc atc   576
Phe Ala Gln Ala Ala Asn Leu His Leu Ser Phe Ile Arg Asp Val Ile
            180                 185                 190

ctt aat gca gat gaa tgg ggc att tca gca gca aca gta cgc aca tat   624
Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Val Arg Thr Tyr
        195                 200                 205

aga gat cac ctg aga aat ttc aca aga gat tac tct aat tat tgt ata   672
Arg Asp His Leu Arg Asn Phe Thr Arg Asp Tyr Ser Asn Tyr Cys Ile
    210                 215                 220

aat acg tat caa act gca ttt aga ggt tta aac act cgt tta cac gat   720
Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp
225                 230                 235                 240

atg tta gaa ttt aga aca tat atg ttt tta aat gta ttt gaa tat gtc   768
Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val
                245                 250                 255

tct atc tgg tcg tta ttt aaa tat caa agc ctt cta gta tct tcc ggc   816
Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Leu Val Ser Ser Gly
            260                 265                 270

gct aat tta tat gcg agt ggt agt ggt cca aca caa tca ttt aca gca   864
Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Thr Gln Ser Phe Thr Ala
        275                 280                 285

caa aac tgg cca ttt tta tat tct ctt ttc caa gtt aat tct aat tat   912
Gln Asn Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser Asn Tyr
    290                 295                 300

gta tta aat ggt ttg agt ggt gct agg acc acc att act ttc cct aat   960
Val Leu Asn Gly Leu Ser Gly Ala Arg Thr Thr Ile Thr Phe Pro Asn
305                 310                 315                 320

att ggt ggt ctt ccc ggt tct acc aca act caa aca ttg cat ttt gcg  1008
Ile Gly Gly Leu Pro Gly Ser Thr Thr Thr Gln Thr Leu His Phe Ala
                325                 330                 335

agg att aat tat aga ggt gga gtg tca tct agc cgc ata ggt caa gct  1056
Arg Ile Asn Tyr Arg Gly Gly Val Ser Ser Ser Arg Ile Gly Gln Ala
            340                 345                 350

aat ctt aat caa aac ttt aac att tcc aca ctt ttc aat cct tta caa  1104
Asn Leu Asn Gln Asn Phe Asn Ile Ser Thr Leu Phe Asn Pro Leu Gln
        355                 360                 365

aca ccg ttt att aga agt tgg cta gat tct ggt aca gat cgg gag ggc  1152
Thr Pro Phe Ile Arg Ser Trp Leu Asp Ser Gly Thr Asp Arg Glu Gly
    370                 375                 380
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gtt gcc acc tct aca aac tgg caa tca gga gcc ttt gag aca act tta   1200
Val Ala Thr Ser Thr Asn Trp Gln Ser Gly Ala Phe Glu Thr Thr Leu
385                 390                 395                 400

tta cga ttt agc att ttt tca gct cgt ggt aat tcg aac ttt ttc cca   1248
Leu Arg Phe Ser Ile Phe Ser Ala Arg Gly Asn Ser Asn Phe Phe Pro
                405                 410                 415

gat tat ttt atc cgt aat att tct ggt gtt gtt ggg act att agc aac   1296
Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Val Gly Thr Ile Ser Asn
            420                 425                 430

gca gat tta gca aga cct cta cac ttt aat gaa ata aga gat ata gga   1344
Ala Asp Leu Ala Arg Pro Leu His Phe Asn Glu Ile Arg Asp Ile Gly
        435                 440                 445

acg aca gca gtc gct agc ctt gta aca gtg cat aac aga aaa aat aat   1392
Thr Thr Ala Val Ala Ser Leu Val Thr Val His Asn Arg Lys Asn Asn
    450                 455                 460

atc tat gac act cat gaa aat ggt act atg att cat tta gcg cca aat   1440
Ile Tyr Asp Thr His Glu Asn Gly Thr Met Ile His Leu Ala Pro Asn
465                 470                 475                 480

gac tat aca gga ttt acc gta tct cca ata cat gcc act caa gta aat   1488
Asp Tyr Thr Gly Phe Thr Val Ser Pro Ile His Ala Thr Gln Val Asn
                485                 490                 495

aat caa att cga acg ttt att tcc gaa aaa tat ggt aat cag ggt gat   1536
Asn Gln Ile Arg Thr Phe Ile Ser Glu Lys Tyr Gly Asn Gln Gly Asp
            500                 505                 510

tcc ttg aga ttt gag cta agc aac aca acg gct cga tac aca ctt aga   1584
Ser Leu Arg Phe Glu Leu Ser Asn Thr Thr Ala Arg Tyr Thr Leu Arg
        515                 520                 525

ggg aat gga aat agt tac aat ctt tat tta aga gta tct tca ata gga   1632
Gly Asn Gly Asn Ser Tyr Asn Leu Tyr Leu Arg Val Ser Ser Ile Gly
    530                 535                 540

agt tcc aca att cga gtt act ata aac ggt aga gtt tat act gca aat   1680
Ser Ser Thr Ile Arg Val Thr Ile Asn Gly Arg Val Tyr Thr Ala Asn
545                 550                 555                 560

gtt aat act acc aca aat aat gat gga gta ctt gat aat gga gct cgt   1728
Val Asn Thr Thr Thr Asn Asn Asp Gly Val Leu Asp Asn Gly Ala Arg
                565                 570                 575

ttt tca gat att aat atc ggt aat gta gtg gca agt gct aat act aat   1776
Phe Ser Asp Ile Asn Ile Gly Asn Val Val Ala Ser Ala Asn Thr Asn
            580                 585                 590

gta cca tta gat ata caa gtg aca ttt aac gac aat cca caa ttt gag   1824
Val Pro Leu Asp Ile Gln Val Thr Phe Asn Asp Asn Pro Gln Phe Glu
        595                 600                 605
```

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ctt atg aat att atg ttg ttc caa cta atc ttc cac cac ttt att aag   1872
Leu Met Asn Ile Met Leu Phe Gln Leu Ile Phe His His Phe Ile Lys
    610                 615                 620

gtt tga                                                          1878
Val
625


<210> 48
<211> 625
<212> PRT
<213> Bacillus thuringiensis

<400> 48
Met Asn Thr Val Leu Asn Asn Gly Arg Asn Thr Thr Cys His Ala His
  1               5                  10                  15
Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asn
            20                  25                  30
Thr Ile Glu Lys Glu Trp Lys Glu Trp Lys Arg Thr Asp His Ser Leu
        35                  40                  45
Tyr Val Ala Pro Ile Val Gly Thr Val Gly Ser Phe Leu Leu Lys Lys
    50                  55                  60
Val Gly Ser Leu Val Gly Lys Arg Ile Leu Ser Glu Leu Gln Asn Leu
65                  70                  75                  80
Ile Phe Pro Ser Gly Ser Ile Asp Leu Met Gln Glu Ile Leu Arg Ala
                85                  90                  95
Thr Glu Gln Phe Ile Asn Gln Arg Leu Asn Ala Asp Thr Leu Gly Arg
            100                 105                 110
Val Asn Ala Glu Leu Ala Gly Leu Gln Ala Asn Val Ala Glu Phe Asn
        115                 120                 125
Arg Gln Val Asp Asn Phe Leu Asn Pro Asn Gln Asn Pro Val Pro Leu
    130                 135                 140
Ala Ile Ile Asp Ser Val Asn Thr Leu Gln Gln Leu Phe Leu Ser Arg
145                 150                 155                 160
Leu Pro Gln Phe Gln Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
                165                 170                 175
Phe Ala Gln Ala Ala Asn Leu His Leu Ser Phe Ile Arg Asp Val Ile
            180                 185                 190
Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Val Arg Thr Tyr
        195                 200                 205
Arg Asp His Leu Arg Asn Phe Thr Arg Asp Tyr Ser Asn Tyr Cys Ile
    210                 215                 220
Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp
225                 230                 235                 240
Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val
                245                 250                 255
Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Leu Val Ser Ser Gly
            260                 265                 270
Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Thr Gln Ser Phe Thr Ala
        275                 280                 285
Gln Asn Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser Asn Tyr
    290                 295                 300
Val Leu Asn Gly Leu Ser Gly Ala Arg Thr Thr Ile Thr Phe Pro Asn
305                 310                 315                 320
Ile Gly Gly Leu Pro Gly Ser Thr Thr Thr Gln Thr Leu His Phe Ala
                325                 330                 335
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Arg Ile Asn Tyr Arg Gly Gly Val Ser Ser Ser Arg Ile Gly Gln Ala
340 345 350
Asn Leu Asn Gln Asn Phe Asn Ile Ser Thr Leu Phe Asn Pro Leu Gln
355 360 365
Thr Pro Phe Ile Arg Ser Trp Leu Asp Ser Gly Thr Asp Arg Glu Gly
370 375 380
Val Ala Thr Ser Thr Asn Trp Gln Ser Gly Ala Phe Glu Thr Thr Leu
385 390 395 400
Leu Arg Phe Ser Ile Phe Ser Ala Arg Gly Asn Ser Asn Phe Phe Pro
405 410 415
Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Val Gly Thr Ile Ser Asn
420 425 430
Ala Asp Leu Ala Arg Pro Leu His Phe Asn Glu Ile Arg Asp Ile Gly
435 440 445
Thr Thr Ala Val Ala Ser Leu Val Thr Val His Asn Arg Lys Asn Asn
450 455 460
Ile Tyr Asp Thr His Glu Asn Gly Thr Met Ile His Leu Ala Pro Asn
465 470 475 480
Asp Tyr Thr Gly Phe Thr Val Ser Pro Ile His Ala Thr Gln Val Asn
485 490 495
Asn Gln Ile Arg Thr Phe Ile Ser Glu Lys Tyr Gly Asn Gln Gly Asp
500 505 510
Ser Leu Arg Phe Glu Leu Ser Asn Thr Thr Ala Arg Tyr Thr Leu Arg
515 520 525
Gly Asn Gly Asn Ser Tyr Asn Leu Tyr Leu Arg Val Ser Ser Ile Gly
530 535 540
Ser Ser Thr Ile Arg Val Thr Ile Asn Gly Arg Val Tyr Thr Ala Asn
545 550 555 560
Val Asn Thr Thr Thr Asn Asn Asp Gly Val Leu Asp Asn Gly Ala Arg
565 570 575
Phe Ser Asp Ile Asn Ile Gly Asn Val Val Ala Ser Ala Asn Thr Asn
580 585 590
Val Pro Leu Asp Ile Gln Val Thr Phe Asn Asp Asn Pro Gln Phe Glu
595 600 605
Leu Met Asn Ile Met Leu Phe Gln Leu Ile Phe His His Phe Ile Lys
610 615 620
Val
625

<210> 49
<211> 143
<212> DNA
<213> Bacillus thuringiensis

<220>
<221> modified_base
<222> (8)..(140)
<223> N = A, T, C or G

<400> 49
gtcgtganag gnccaggatt tacaggaggg gatatactnc gaagaacggn cggtggtgca 60
tttggaacna ttagngctan ggctantgcc ccnttaacac aacaatatcg nataagatta 120
cgctntgctt ctacnacaan ttt 143

<210> 50
<211> 47
<212> PRT
<213> Bacillus thuringiensis

<220>
<221> SITE
<222> (3)
<223> X = R, I, K, or T

<220>
<221> SITE
<222> (17)
<223> X = A, D, G or V

<220>
<221> SITE
<222> (25)
<223> X = S, or R

<220>
<221> SITE
<222> (27)
<223> X = R, K, M, or T

<220>
<221> SITE
<222> (29)
<223> X = N, I, S, or T

<220>
<221> SITE
<222> (42)
<223> X = C, F, S, or Y

<220>
<221> SITE
<222> (47)
<223> X = N, I, S, or T

<400> 50
Val Val Xaa Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr
1 5 10 15

Xaa Gly Gly Ala Phe Gly Thr Ile Xaa Ala Xaa Ala Xaa Ala Pro Leu
20 25 30

Thr Gln Gln Tyr Arg Ile Arg Leu Arg Xaa Ala Ser Thr Thr Xaa
35 40 45

<210> 51
<211> 42
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 51
tggatacttg atcaatatga taatccgtca catctgtttt ta 42

<210> 52
<211> 61
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 52
agtaacggtg ttactattag cgagggcggt ccattcttta aggtcgtgca cttcagttag 60
c 61

<210> 53
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 53
cgacttctcc tgctaatgga gg 22

<210> 54
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 54
ctcgctaata gtaacaccgt tacttgcc 28

<210> 55
<211> 61
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 55
atttagtagc atgcgttgca ctttgtgcat tttttcataa gatgagtcat atgttttaaa 60
t 61

<210> 56

<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 56
ggatagcact catcaaaggt acc 23

<210> 57
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 57
gtwtggacsc rtcghgatgt gg 22

<210> 58
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 58
taatttctgc tagcccwatt tctggattta attgttgatc 40

<210> 59
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (3)..(12)
<223> W = A, T

<220>
<221> modified_base
<222> (6)
<223> N = A, C, T

<220>
<221> modified_base
<222> (10)
<223> M = A, C

<220>
<221> modified_base

<222> (18)
<223> R = A, G

<220>
<221> modified_base
<222> (15)
<223> D = A, G

<220>
<223> Description of Artificial Sequence: primer

<400> 59
atwacncaam twccdttrg 19

<210> 60
<211> 17
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: primer

<400> 60
aatgcagatg aatgggg 17

<210> 61
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 61
tgataatgga gctcgtt 17

<210> 62
<211> 3684
<212> DNA
<213> Bacillus thuringiensis
<400> 62
ttgacttcaa ataggaaaaa tgagaatgaa attataaatg ctttatcgat tccagctgta 60
tcgaatcatt ccgcacaaat gaatctatca accgatgctc gtattgagga tagcttgtgt 120
atagccgagg ggaacaatat cgatccattt gttagcgcat caacagtcca aacgggtatt 180
aacatagctg gtagaatact aggtgtatta ggcgtaccgt ttgctggaca aatagctagt 240
ttttatagtt ttcttgttgg tgaattatgg ccccgcggca gagatccttg ggaaattttc 300
ctagaacatg tcgaacatct tataagacaa caagtaacag aaaatactag ggatacggct 360
cttgctcgat tacaaggttt aggaaattcc tttagagcct atcaacagtc acttgaagat 420
tggctagaaa accgtgatga tgcaagaacg agaagtgttc tttataccca atatatagcc 480
ttagaacttg attttcttaa tgcgatgccg cttttcgcaa ttagaaacca agaagttcca 540
ttattaatgg tatatgctca agctgcaaat ttacacctat tattattgag agatgcctct 600
ctttttggta gtgaatttgg gcttacatcc caagaaattc aacgttatta tgagcgccaa 660
gtggaaaaaa cgagagaata ttctgattat tgcgcaagat ggtataatac gggtttaaat 720
aatttgagag ggacaaatgc tgaaagttgg ttgcgatata atcaattccg tagagactta 780
acgctaggag tattagatct agtggcacta ttcccaagct atgacacgcg tgtttatcca 840

atgaatacca gtgctcaatt aacaagagaa atttatacag atccaattgg gagaacaaat 900
gcaccttcag gatttgcaag tacgaattgg tttaataata atgcaccatc gttttctgcc 960
atagaggctg ccgttattag gcctccgcat ctacttgatt ttccagaaca gcttacaatt 1020
ttcagcgtat taagtcgatg gagtaatact caatatatga attactgggt gggacataga 1080
cttgaatcgc gaacaataag ggggtcatta agtacctgga cacacggaaa taccaatact 1140
tctattaatc ctgtaacatt acagttcaca tctcgagacg tttatagaac agaatcattt 1200
gcagggataa atatacttct aactactcct gtgaatggag taccttgggc tagatttaat 1260
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gtggggacac aactatttga ttcagaaact gaattaccac cagaaacaac agaacgacca 1380
aattatgaat cttacagtca tagattatct aatataagac taatatcagg aaacactttg 1440
agagcaccag tatattcttg gacgcaccgt agtgcagatc gtacaaatac cattagttca 1500
gatagcataa cacaaatacc attggtaaaa tcattcaacc ttaattcagg tacctctgta 1560
gtcagtggcc caggatttac aggaggggat ataatccgaa ctaacgttaa tggtagtgta 1620
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tatgctgctt ctcaaacaat ggtcctgagg gtaactgtcg gagggagtac tacttttgat 1740
caaggattcc ctagtactat gagtgcaaat gagtctttga catctcaatc atttagattt 1800
gcagaatttc ctgtaggtat tagtgcatct ggcagtcaaa ctgctggaat aagtataagt 1860
aataatgcag gtagacaaac gtttcacttt gataaaattg aattcattcc aattactgca 1920
accttcgaag cagaatatga tttagaaaga gcgcaagagg cggtgaatgc tctgtttact 1980
aatacgaatc caagaaggtt gaaaacaggt gtgacagatt atcatattga tgaagtatcc 2040
aatttagtgg cgtgtttatc ggatgaattc tgcttggatg aaaagagaga attacttgag 2100
aaagtgaaat atgcgaaacg actcagtgat gaaagaaact tactccaaga tccaaacttc 2160
acatccatca ataagcaacc agacttcaat tctaataatg agcaatcgaa tttcacatct 2220
atccatgaac aatctgaaca tggatggtgg ggaagtgaga acattacaat ccaggaagga 2280
aatgacgtat ttaaagagaa ttacgtcaca ctaccgggta cttttaatga gtgttatccg 2340
acgtatttat atcaaaaaat aggggaggcg gaattaaaag cttatactcg ctaccaatta 2400
agtggctata ttgaagatag tcaagattta gagatatatt tgattcgtta caatgcgaaa 2460
catgaaacat tggatgttcc aggtaccgag tccgtatggc cgctttcagt tgaaagccca 2520
atcggaaggt gcggagaacc gaatcgatgc gcaccacatt ttgaatggaa tcctgatcta 2580
gattgttcct gcagagatgg agaaaaatgt gcgcatcatt cccatcattt ctctttggat 2640
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ttaggagaag cactgtctcg tgtgaagaga gcagagaaaa aatggagaga caaacgtgaa 2820
aaactacaat tggaaacaaa acgagtatat acagaggcaa aagaagctgt ggatgcttta 2880
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gcagataaac ttgttcatcg aattcgagag gcgtatcttt cagaattatc tgttatccca 3000
ggtgtaaatg cggaaatttt tgaagaatta gaaggtcgca ttatcactgc aatctcccta 3060
tacgatgcga gaaatgtcgt taaaaatggt gattttaata atggattagc atgctggaat 3120
gtaaaagggc atgtagatgt acaacagagc catcaccgtt ctgtccttgt tatcccagaa 3180
tgggaagcag aagtgtcaca agcagttcgc gtctgtccgg ggcgtggcta tatcctccgt 3240
gtcacagcgt acaaagaggg atatggagag ggttgtgtaa ctatccatga aatcgagaac 3300
aatacagacg aactaaaatt taaaaactgt gaagaagagg aagtgtatcc aacggataca 3360
ggaacgtgta atgattatac tgcacaccaa ggtacagcag tatgtaattc ccgtaatgct 3420
ggatatgagg atgcatatga agttgatact acagcatctg ttaattacaa accgacttat 3480
gaagaagaaa cgtatacaga tgtacgaaga gataatcatt gtgaatatga cagagggtat 3540
gtgaattatc caccagtacc agctggttat atgacaaaag aattagaata cttcccagaa 3600
accgataagg tatggattga gattggagaa acggaaggga agtttattgt agacagcgtg 3660
gaattactcc ttatggagga atag 3684

<210> 63
<211> 1227
<212> PRT
<213> Bacillus thuringiensis
<400> 63
Leu Thr Ser Asn Arg Lys Asn Glu Asn Glu Ile Ile Asn Ala Leu Ser
1 5 10 15

Ile Pro Ala Val Ser Asn His Ser Ala Gln Met Asn Leu Ser Thr Asp
20 25 30

Ala Arg Ile Glu Asp Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asp
35 40 45

Pro Phe Val Ser Ala Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly
50 55 60

Arg Ile Leu Gly Val Leu Gly Val Pro Phe Ala Gly Gln Ile Ala Ser
65 70 75 80

Phe Tyr Ser Phe Leu Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Pro
85 90 95

Trp Glu Ile Phe Leu Glu His Val Glu His Leu Ile Arg Gln Gln Val
100 105 110

Thr Glu Asn Thr Arg Asp Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly
115 120 125

Asn Ser Phe Arg Ala Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn
130 135 140

Arg Asp Asp Ala Arg Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala
145 150 155 160

Leu Glu Leu Asp Phe Leu Asn Ala Met Pro Leu Phe Ala Ile Arg Asn
165 170 175

Gln Glu Val Pro Leu Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His
180 185 190

Leu Leu Leu Leu Arg Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu
195 200 205

Thr Ser Gln Glu Ile Gln Arg Tyr Tyr Glu Arg Gln Val Glu Lys Thr
210 215 220

Arg Glu Tyr Ser Asp Tyr Cys Ala Arg Trp Tyr Asn Thr Gly Leu Asn
225 230 235 240

Asn Leu Arg Gly Thr Asn Ala Glu Ser Trp Leu Arg Tyr Asn Gln Phe
245 250 255

Arg Arg Asp Leu Thr Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro
260 265 270

Ser Tyr Asp Thr Arg Val Tyr Pro Met Asn Thr Ser Ala Gln Leu Thr
275 280 285

Arg Glu Ile Tyr Thr Asp Pro Ile Gly Arg Thr Asn Ala Pro Ser Gly
290 295 300

Phe Ala Ser Thr Asn Trp Phe Asn Asn Asn Ala Pro Ser Phe Ser Ala
305 310 315 320

```
Ile Glu Ala Ala Val Ile Arg Pro Pro His Leu Leu Asp Phe Pro Glu
                325                 330                 335

Gln Leu Thr Ile Phe Ser Val Leu Ser Arg Trp Ser Asn Thr Gln Tyr
            340                 345                 350

Met Asn Tyr Trp Val Gly His Arg Leu Glu Ser Arg Thr Ile Arg Gly
        355                 360                 365

Ser Leu Ser Thr Trp Thr His Gly Asn Thr Asn Thr Ser Ile Asn Pro
    370                 375                 380

Val Thr Leu Gln Phe Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Phe
385                 390                 395                 400

Ala Gly Ile Asn Ile Leu Leu Thr Thr Pro Val Asn Gly Val Pro Trp
                405                 410                 415

Ala Arg Phe Asn Trp Arg Asn Pro Leu Asn Ser Leu Arg Gly Ser Leu
            420                 425                 430

Leu Tyr Thr Ile Gly Tyr Thr Gly Val Gly Thr Gln Leu Phe Asp Ser
        435                 440                 445

Glu Thr Glu Leu Pro Pro Glu Thr Thr Glu Arg Pro Asn Tyr Glu Ser
    450                 455                 460

Tyr Ser His Arg Leu Ser Asn Ile Arg Leu Ile Ser Gly Asn Thr Leu
465                 470                 475                 480

Arg Ala Pro Val Tyr Ser Trp Thr His Arg Ser Ala Asp Arg Thr Asn
                485                 490                 495

Thr Ile Ser Ser Asp Ser Ile Thr Gln Ile Pro Leu Val Lys Ser Phe
            500                 505                 510

Asn Leu Asn Ser Gly Thr Ser Val Val Ser Gly Pro Gly Phe Thr Gly
        515                 520                 525

Gly Asp Ile Ile Arg Thr Asn Val Asn Gly Ser Val Leu Ser Met Gly
    530                 535                 540

Leu Asn Phe Asn Asn Thr Ser Leu Gln Arg Tyr Arg Val Arg Val Arg
545                 550                 555                 560

Tyr Ala Ala Ser Gln Thr Met Val Leu Arg Val Thr Val Gly Gly Ser
                565                 570                 575

Thr Thr Phe Asp Gln Gly Phe Pro Ser Thr Met Ser Ala Asn Glu Ser
            580                 585                 590

Leu Thr Ser Gln Ser Phe Arg Phe Ala Glu Phe Pro Val Gly Ile Ser
        595                 600                 605

Ala Ser Gly Ser Gln Thr Ala Gly Ile Ser Ile Ser Asn Asn Ala Gly
    610                 615                 620
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Arg Gln Thr Phe His Phe Asp Lys Ile Glu Phe Ile Pro Ile Thr Ala
625 630 635 640

Thr Phe Glu Ala Glu Tyr Asp Leu Glu Arg Ala Gln Glu Ala Val Asn
645 650 655

Ala Leu Phe Thr Asn Thr Asn Pro Arg Arg Leu Lys Thr Gly Val Thr
660 665 670

Asp Tyr His Ile Asp Glu Val Ser Asn Leu Val Ala Cys Leu Ser Asp
675 680 685

Glu Phe Cys Leu Asp Glu Lys Arg Glu Leu Leu Glu Lys Val Lys Tyr
690 695 700

Ala Lys Arg Leu Ser Asp Glu Arg Asn Leu Leu Gln Asp Pro Asn Phe
705 710 715 720

Thr Ser Ile Asn Lys Gln Pro Asp Phe Asn Ser Asn Asn Glu Gln Ser
725 730 735

Asn Phe Thr Ser Ile His Glu Gln Ser Glu His Gly Trp Trp Gly Ser
740 745 750

Glu Asn Ile Thr Ile Gln Glu Gly Asn Asp Val Phe Lys Glu Asn Tyr
755 760 765

Val Thr Leu Pro Gly Thr Phe Asn Glu Cys Tyr Pro Thr Tyr Leu Tyr
770 775 780

Gln Lys Ile Gly Glu Ala Glu Leu Lys Ala Tyr Thr Arg Tyr Gln Leu
785 790 795 800

Ser Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu Ile Tyr Leu Ile Arg
805 810 815

Tyr Asn Ala Lys His Glu Thr Leu Asp Val Pro Gly Thr Glu Ser Val
820 825 830

Trp Pro Leu Ser Val Glu Ser Pro Ile Gly Arg Cys Gly Glu Pro Asn
835 840 845

Arg Cys Ala Pro His Phe Glu Trp Asn Pro Asp Leu Asp Cys Ser Cys
850 855 860

Arg Asp Gly Glu Lys Cys Ala His His Ser His His Phe Ser Leu Asp
865 870 875 880

Ile Asp Val Gly Cys Ile Asp Leu His Glu Asn Leu Gly Val Trp Val
885 890 895

Val Phe Lys Ile Lys Thr Gln Glu Gly His Ala Arg Leu Gly Asn Leu
900 905 910

Glu Phe Ile Glu Glu Lys Pro Leu Leu Gly Glu Ala Leu Ser Arg Val
915 920 925

```
Lys Arg Ala Glu Lys Lys Trp Arg Asp Lys Arg Glu Lys Leu Gln Leu
    930                 935                 940

Glu Thr Lys Arg Val Tyr Thr Glu Ala Lys Glu Ala Val Asp Ala Leu
945                 950                 955                 960

Phe Val Asp Ser Gln Tyr Asp Arg Leu Gln Ala Asp Thr Asn Ile Gly
                965                 970                 975

Met Ile His Ala Ala Asp Lys Leu Val His Arg Ile Arg Glu Ala Tyr
            980                 985                 990

Leu Ser Glu Leu Ser Val Ile Pro Gly Val Asn Ala Glu Ile Phe Glu
        995                1000                1005

Glu Leu Glu Gly Arg Ile Ile Thr Ala Ile Ser Leu Tyr Asp Ala Arg
   1010                1015                1020

Asn Val Val Lys Asn Gly Asp Phe Asn Asn Gly Leu Ala Cys Trp Asn
1025                1030                1035                1040

Val Lys Gly His Val Asp Val Gln Gln Ser His His Arg Ser Val Leu
                1045                1050                1055

Val Ile Pro Glu Trp Glu Ala Glu Val Ser Gln Ala Val Arg Val Cys
            1060                1065                1070

Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr Ala Tyr Lys Glu Gly Tyr
        1075                1080                1085

Gly Glu Gly Cys Val Thr Ile His Glu Ile Glu Asn Asn Thr Asp Glu
   1090                1095                1100

Leu Lys Phe Lys Asn Cys Glu Glu Glu Glu Val Tyr Pro Thr Asp Thr
1105                1110                1115                1120

Gly Thr Cys Asn Asp Tyr Thr Ala His Gln Gly Thr Ala Val Cys Asn
                1125                1130                1135

Ser Arg Asn Ala Gly Tyr Glu Asp Ala Tyr Glu Val Asp Thr Thr Ala
            1140                1145                1150

Ser Val Asn Tyr Lys Pro Thr Tyr Glu Glu Glu Thr Tyr Thr Asp Val
        1155                1160                1165

Arg Arg Asp Asn His Cys Glu Tyr Asp Arg Gly Tyr Val Asn Tyr Pro
   1170                1175                1180

Pro Val Pro Ala Gly Tyr Met Thr Lys Glu Leu Glu Tyr Phe Pro Glu
1185                1190                1195                1200

Thr Asp Lys Val Trp Ile Glu Ile Gly Glu Thr Glu Gly Lys Phe Ile
                1205                1210                1215

Val Asp Ser Val Glu Leu Leu Leu Met Glu Glu
            1220                1225
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10 May 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/19859 A3

(54) Title: LEPIDOPTERAN-ACTIVE *BACILLUS THURINGIENSIS* δ-ENDOTOXIN COMPOSITIONS AND METHODS OF USE

(57) Abstract: Disclosed are *Bacillus thuringiensis* strains comprising novel crystal proteins which exhibit insecticidal activity against lepidopteran insects. Also disclosed are novel *B. thuringiensis* genes and their encoded crystal proteins, as well as methods of making and using transgenic cells comprising the novel nucleic acid sequences of the invention.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/25361

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/32 C07K14/325 A01N63/00 C12N15/63 C12N5/10
C12N15/82 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 338 544 A (DONOVAN) 16 August 1994 (1994-08-16) abstract; figures 2A-C column 3, line 19 - line 40	2,6-11
X A	US 5 723 758 A (CANNON RAYMOND J C ET AL) 3 March 1998 (1998-03-03) cited in the application Seq Id Nos 7,8 column 1, line 13 - line 57 column 2, line 24 - line 38 examples 2,3	2,6-11, 23-27, 34-51 1-11, 14-51

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 June 2001	20. 6. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ceder, O

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KUO ET AL.: "Bacillus thuringiensis wuhanensis insecticidal crystal protein CryE1 (cryLa1) gene, complete cds." EMBL SEQUENCE DATABASE, 6 January 1999 (1999-01-06), XP002160714 HEIDELBERG DE Ac U70726 the whole document	1-6, 14-17
A	WO 95 06128 A (DEKALB GENETICS CORP) 2 March 1995 (1995-03-02) abstract; claims 11-14,22-25 Seq Id Nos 10, 11 page 6, line 1 -page 9, line 3 page 19, line 20 - line 25 page 29, line 6 - line 25 page 74, line 16 - line 27 page 129, line 10 -page 130, line 10 page 243, line 15 -page 244, line 6	1-6, 14-20, 22-26, 30-32, 34-38, 41-45, 47-51
A	EP 0 367 474 A (MYCOGEN CORP) 9 May 1990 (1990-05-09) the whole document	1-11, 14-51
A	EP 0 206 613 A (REPLIGEN CORP) 30 December 1986 (1986-12-30) the whole document	1-11, 14-51
A	HOFTE H ET AL: "INSECTICIDAL CRYSTAL PROTEINS OF BACILLUS THURINGIENSIS" MICROBIOLOGICAL REVIEWS,US,AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, DC, vol. 53, 1 June 1989 (1989-06-01), pages 242-255, XP000374163 ISSN: 0146-0749 cited in the application	
A	WO 98 00546 A (MYCOGEN CORP) 8 January 1998 (1998-01-08) abstract; claims 21,22; example 13 page 16, line 10 -page 17, line 10	1-11, 14-51
A	WO 98 23641 A (ECOGEN INC) 4 June 1998 (1998-06-04) page 10, line 14 -page 14, line 20	1-11, 14-51

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/25361

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1-11, 14-51 all partially (Inventions 1,6,10 searched)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-11, 14-51 all partially

   Isolated polypeptide, polynucleotide encoding it and composition containing it; vector, transformed host cell, plant and its progeny and seeds containing the polynucleotide; methods of controlling Lepdopteran insects and for preparing insect resistant plants using the above polypeptide and polynucleotide; the polynucleotide and polypeptide being Seq Id Nos 17 and 18, respectively.

2. Claims: 1-11, 14-51 all partially

   Subject matter as defined for invention 1 above, but limited to Seq Id Nos 19 and 20.

3. Claims: 1-11, 14-51 all partially

   Subject matter as defined for invention 1 above, but limited to Seq Id Nos 23 and 24.

4. Claims: 1-11, 14-51 all partially

   Subject matter as defined for invention 1 above, but limited to Seq Id Nos 39 and 40.

5. Claims: 1-11, 14-51 all partially

   Subject matter as defined for invention 1 above, but limited to Seq Id Nos 43 and 44.

6. Claims: 2-11, 15-51 all partially

   Subject matter as defined for invention 1 above, but limited to Seq Id Nos 1 and 2.

7. Claims: 2-11, 15-51 all partially

   Subject matter as defined for invention 1 above, but limited to Seq Id Nos 5 and 6.

8. Claims: 2-11, 15-51 all partially

   Subject matter as defined for invention 1 above, but limited to Seq Id Nos 7 and 8.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

9. Claims: 2-11, 15-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 9 and 10.

10. Claims: 2-11, 15-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 37 and 38.

11. Claims: 3-11, 15-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 15 and 16.

12. Claims: 3-11, 15-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 21 and 22.

13. Claims: 3-11, 15-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 27 and 28.

14. Claims: 3-11, 15-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 35 and 36.

15. Claims: 3-11, 15-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 49 and 50.

16. Claims: 4-11, 16-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 3 and 4.

17. Claims: 4-11, 16-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 11 and 12.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

18. Claims: 4-11, 16-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 13 and 14.

19. Claims: 4-11, 16-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 25 and 26.

20. Claims: 4-11, 16-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 29 and 30.

21. Claims: 4-11, 16-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 31 and 32.

22. Claims: 4-11, 16-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 33 and 34.

23. Claims: 4-11, 16-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 41 and 42.

24. Claims: 4-11, 15-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 45 and 46.

25. Claims: 4-11, 16-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 47 and 48.

26. Claims: 4-11, 15-51 all partially

Subject matter as defined for invention 1 above, but limited to Seq Id Nos 62 and 63.

27. Claims: 12, 13 both partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A Bacillus thuringiensis cell and an insecticidal polypeptide prepared from it, where the cell is NRRL B-21784.

28. Claims: 12, 13 both partially

Subject matter as defined for invention 27 above, but limited to NRRL B-21783.

29. Claims: 12, 13 both partially

Subject matter as defined for invention 27 above, but limited to NRRL B-21917.

30. Claims: 12, 13 both partially

Subject matter as defined for invention 27 above, but limited to NRRL B-21786

31. Claims: 12, 13 both partially

Subject matter as defined for invention 27 above, but limited to NRRL B-21787.

32. Claims: 12, 13 both partially

Subject matter as defined for invention 27 above, but limited to NRRL B-21785.

33. Claims: 12, 13 both partially

Subject matter as defined for invention 27 above, but limited to NRRL B-21788.

34. Claims: 12, 13 both partially

Subject matter as defined for invention 27 above, but limited to NRRL B-21915.

35. Claims: 12, 13 both partially

Subject matter as defined for invention 27 above, but limited to NRRL B-21916.

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Information on patent family members

International Application No
PCT/US 00/25361

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